National Institute
of
Allergy and Infectious
Diseases

# Annual Report of Intramural Activities

October 1, 1984 - September 30, 1985

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

National Institute
of
Allergy and Infectious
Diseases (U.S.

# Annual Report of Intramural Activities

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**U.S. Department of Health and Human Services**
Public Health Service
National Institutes of Health

**For Administrative Use**

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1985 ANNUAL REPORT OF INTRAMURAL ACTIVITIES

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1985 ANNUAL REPORT PROJECT NUMBER LISTING*

Z01 AI
00011-20 LMM
00013-22 LMM
00020-10 LVD
00027-18 LMM
00030-17 LI
00035-10 LI
00036-20 LI
00043-20 LCI
00045-17 LCI
00047-16 LCI
00048-15 LCI
00057-12 LCI
00058-11 LCI
00061-23 LPB
00063-15 LPB
00065-12 LMSF
00071-14 LPB
00072-14 LPVD
00074-13 LPVD
00082-24 LPB
00085-08 LPVD
00086-08 LPVD
00094-26 LPD
00097-27 LPD
00098-29 LPD
00099-15 LPD
00102-11 LPD
00103-18 LPD
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00131-18 LMI
00134-23 LMI
00135-11 LIP
00136-13 LMI
00138-11 LIP
00143-16 LMI
00144-21 LMI
00145-18 LMI
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00148-10 LI
00153-08 LMI
00154-10 LCI
00155-10 LCI
00161-08 LPD

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00168-08 LIG
00169-08 LIG
00170-08 LIG
00171-08 LIG
00172-07 OSD
00173-08 LIG
00180-07 LIG
00182-07 OSD
00183-07 OSD
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00190-07 LMM
00192-07 LCI
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00197-06 LPD
00199-06 LPVD
00201-06 LPB
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00213-05 LIR
00216-05 LMSF
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00226-04 LI
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00251-04 LPD

*Does not include terminated or inactive projects

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1985 ANNUAL REPORT PROJECT NUMBER LISTING

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00389-02 LIG

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1985 ANNUAL REPORT PROJECT NUMBER LISTING

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00460-01 LID
00461-01 LID
00462-01 LID
00463-01 LID

OFFICE OF THE SCIENTIFIC DIRECTOR, NIAID
1985 Annual Report
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Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1984 - September 30, 1985

The Intramural Research Program (IRP) of the National Institute of Allergy and Infectious Diseases consists of 14 laboratories. The individual research summaries describing the research in these laboratories are contained in the following pages. Eleven of the laboratories are located at the NIH campus in Bethesda, while the remaining three laboratories are located at the Rocky Mountain Laboratories facility in Hamilton, Montana. The Office of the Scientific Director (OSD) is responsible for the administrative management of the Intramural Research Program, both in Montana and Bethesda.

Effective in February, 1985, the Scientific Director, Kenneth Sell, M.D., Ph.D., left the OSD to assume responsibility as Chairman, Department of Pathology, Emory University Medical School, Atlanta, Georgia. The Acting Scientific Director during this interim period has been Gordon Wallace, D.V.M. The organizational structure of the OSD has been changed to include an Animal Care Branch and an Administrative Branch. When feasible, scientific and technical personnel assigned to OSD have been reassigned to appropriate laboratories.

Considerable IRP resources have been dedicated to AIDS research, the HHS number one health priority. Scientists in LIR have been in the forefront in gaining knowledge on pathogenesis and in the search for effective therapies for the disease and its associated opportunistic infections. They have found in preliminary trials that interleukin 2 can cause regression of AIDS associated Kaposi's sarcoma. Further studies will determine the optimal dosage and course to follow in treatment. Attempts at restoration of immune function in AIDS patients by bone marrow transplants from a healthy twin is also in progress. Antiviral drugs, such as Suramin and Ribavirin, are being studied and evaluated. An effective treatment for AIDS may require the combined use of antivirals and immunotherapy.

NIAID scientists, in LMM and LIR, have been studying the effect of HTLV III/LAV virus on lymphocytes. They have developed a cell line in which the AIDS virus propagates and are following events in the infected cell leading to cell death, or survival. They have studied biological variations of individual AIDS retrovirus isolates and are gaining an understanding of how cellular genes control and modify the virus production.

NIAID-IRP remains a world leader in basic immunologic research. Scientists in LI, LIR, LCI, LIG and LMI have made immense contributions to the understanding of the structure, function and regulation of the immune system. Clinical and basic research in LCI and LIR have significantly contributed to understanding the pathogenesis of immunologic diseases and provided rational and effective interventions in certain disorders, including allergic disease.

In concert with basic research on infectious agents in NIAID laboratories, new and exciting vaccine candidates are being developed. Hepatitis A virus (HAV) has been molecularly cloned and sequenced by scientists in LID, providing the opportunity to determine the molecular basis for attenuation and virulence. The NIH hepatitis B vaccine, developed in LID several years ago, has been shown recently to be highly effective in preventing type B hepatitis infection in newborns in China. A rotavirus vaccine to prevent a major cause of diarrheal illness in infants and young children has recently been developed in LID and is undergoing field trials. At RML, scientists have cloned pertussis toxin genes, paving the way for an improved pertussis vaccine. The use of vaccinia virus as an expression vector to incorporate genes from other infectious agents that will be expressed as antigenic proteins has provided an exciting new approach to live vaccines. In LBV, vaccines developed by this system are being tested for effectiveness against hepatitis B, herpes simplex, vesicular stomatitis, respiratory syncytial viruses, rotavirus, malaria and the AIDS retrovirus.

The Malaria Section, LPD, isolated and cloned a biologically active malaria gene coding for the dominant protein on the surface of the malaria sporozoite, thought to be responsible for immunity to this stage. The Section further contributed to research leading to the biological production of the protein in *E. coli*. With the aid of collaborators, the protein has been extensively tested in animals, found to be nontoxic and to produce high levels of antibody. Human trials will be in process soon, remarkably less than two years following cloning of the gene.

Collaborators in NIAID's Rocky Mountain Laboratories have shown that the prion protein associated with scrapie is probably a component of normal healthy brain tissue. These results cast doubt on the speculation that the prion-protein is a self-replicating protein.

Equal Employment Opportunity and Affirmative Action Programs have been given considerable attention by OSD during the past year. Conscious efforts to recruit minorities have resulted in the addition of a number of minority staff members and fellows over the past year. The NIAID-IRP Introduction to Biomedical Research Program was held in February as usual. However, due to problems with full time equivalent (FTE) cuts by OPM, only 13 students were supported for summer work. Support was in the form of modest fellowships awarded by FAES. Unless there is a change in FTE limits, the Program will be discontinued next year.

Plans for the demolition and renovation of Building 4, to house NIAID scientists now occupying Building 5, have been completed by the NIH Engineering Design Branch, contract architects and with involvement by NIAID intramural laboratory chiefs affected by the move. It is anticipated that the move will occur in the summer of 1988, at which time Building 5 will be vacated for renovation.

The NIAID Board of Scientific Counselors reviewed the Rocky Mountain Laboratories (RML) during the past year. The high quality of scientific research in the IRP continues to be recognized in these evaluations. The Rocky Mountain Laboratories were particularly complemented for excellent research programs and accomplishments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00417-02OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Systematics and Vector Relationships of Ticks (Ixodoidea)

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: James E. Keirans Research Entomologist OSD, NIAID

COOPERATING UNITS (*if any*)

Dr. H. Hoogstraal, NAMRU-3; Drs. D. Sonenshine and P. Homsher, Old Dominion Univ.; Dr. Jane B. Walker, Div. of Vet. Services, Onderstepoort; Dr. Rupert Pegram, Tick Diseases Unit, Lusaka, Zambia.

LAB/BRANCH

Office of Scientific Director

SECTION

Entomology Department, Museum Support Center, Smithsonian, Wash., DC

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.80 | 1 | 1.80 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This research project currently comprises three main functions: (1) specific identification of ticks received from health professionals, universities, and governmental agencies around the world; (2) systematic investigations of the Ixodoidea, including their taxonomy, classification, life histories, and ecology; and (3) mobilization of our vast data base via the Smithsonian computer system to provide interested individuals with the latest information on any tick species.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00172-07 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Synthesis of Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Walter L. Maloy, Expert, OSD, NIAID Others: John E. Coligan, Research Chemist, LIG, NIAID

COOPERATING UNITS (if any)

Thomas Kindt, LIG, NIAID; Louis Miller, LPD, NIAID; Ronald Schwartz, LI, NIAID; Bahige Baroudy, LID, NIAID; David Margulies, LI, NIAID. Office of the Scientific Director, NIAID

LAB/BRANCH

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

SECTION

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.0 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Within the last year we have synthesized 110 peptides for use in twenty-six separate studies. These studies are run by sixteen investigators within the NIAID, and will be described in detail by those investigators. The majority of the peptides have been used to prepare anti-peptide antisera in rabbits. Anti-peptide antisera reactive with mouse MHC class I molecules have been used to detect the Q8 and Q10 gene products in various tissues and mouse strains. Other sera reactive with C-terminal regions of the H-$2K^b$ molecule have been used to detect alternate RNA splicing patterns in the gene products of the H-$2K^b$ and H-$2D^d$ genes. Finally, sera and a monoclonal reactive with regions of the N domain of the H-$2K^b$ molecule have been used to determine the extent of variability among various H-2 molecules. Anti-peptide sera reactive with MCF and xenotropic type C retroviruses have been used as specific typing reagents for these viruses. Anti-peptide sera reactive with a Hepatitus A virus (HAV) VPg like sequence was used to immunoprecipitate HAV RNA extracted from virions. Anti-peptide sera specific for the alpha and beta chains of the I-A molecule have also been prepared.

In addition to using peptides to make antisera, peptides have also been used to map determinants recognized by antisera made against intact proteins. This approach has been used for monoclonals against the Plasmodium falciparum sporozoite surface antigens and antisera against the Hepatitus B virus. Finally many peptides have been made from sequences contained in cytochrome C and used to define the T-cell epitopes on this molecule.




PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00383-03 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acquisition of Specimens from Cases of Acquired Immune Deficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Lois A. Salzman, Ph.D., OSD/NIAID

COOPERATING UNITS *(if any)*

Dr. Louis Baker, New York Blood Center; Dr. Jonathan Gold, Memorial Sloan-Kettering Cancer Center

LAB/BRANCH

Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.5 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The recent identification of the retrovirus HTLV III/LAV as the probable cause of AIDS has shifted our focus from the causative agent to its control, treatment and prevention. It is now important to define the early and late clinical, virologic, serologic and immunologic events associated with HTLV III/LAV infection. Questions to be answered include a knowledge of long term manifestations of infection with HTLV III/LAV prior to development of diagnosed AIDS and in patients who have antibody to the virus but have not developed defined AIDS or pre-AIDS. The intramural research contract with NYBC and Memorial Sloan-Kettering was designed to collect specimens in a prospective fashion from 325 homosexual males. Three populations were recruited: (1) patients with lymphadenopathy (100), (2) normal plasma donors (175) and (3) normal homosexual males from a geographically distinct area in New York State (50). Specimens of peripheral blood leukocytes, plasma, serum, urine, saliva and stool are being collected at intervals along with epidemiological information. Using these specimens collected over several years, it may be possible to correlate the presence of HTLV III/LAV antibody to clinical disease, to locate and isolate the virus from several sources and to gain information about changes in immune function in participants with antibody to HTLV III/LAV who do or do not develop AIDS.



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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00421-02 OSD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Specimens Collected from Populations at Risk of AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M.J. Waxdal, Senior Investigator OSD/NIAID
Lois Salzman, Contract Officer, OSD/NIAID
Albert Saah, Medical Epidemiologist, ESB/MIDP/NIAID
Richard Kaslow, Chief, EBS/MIDP/NIAID

COOPERATING UNITS *(if any)* Louis Baker, N.Y. Blood Center; Jonathan Gold, Memorial Sloan-Kettering; Roger Detels, UCLA; David Ostrow, Howard Brown Memorial Clinic, Chicago; Frank Polk, Johns Hopkins; Charles Renaldo, U. Pittsburgh; Warren Winkelstin, UC, Berkeley; Jim Leaf, BRI, Rockville, MD.

LAB/BRANCH
Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.6 | 0.6 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


To establish, implement and maintain the Repository Data Center for the joint AIDS contracts effort between the Intramural Research Program (OSD/IRP) and the Microbiology and Infectious Disease Program (MIDP). This effort is a study of the epidemiology, pathogenesis, natural history, susceptibility, and causative agents of Acquired Immune Deficiency Syndrome (AIDS). The data base will contain information on approximately two million medical specimens from six contractors.

Specimens from selected patients will be sent to qualified investigators for special testing and biological research. The data on these removals from the repository, on the tests and research to be performed, and the results also will be entered and maintained in the database.

To coordinate and evaluate flow cytometry studies of patient PBL by the AIDS contractors.




PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00182-07 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical & Genetic Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. C. Williams, Ph.D., Sr. Scientist, NIAID/OSD, Group Leader, Rickettsial Diseases Laboratory, USAMRIID

E. H. Stephenson, DVM, Ph.D., COL, VC, Chief, Airborne Diseases Division, USAMRIID
M. H. Vodkin, Ph.D., USAMRIID
C. E. Snyder, Jr., Ph.D., CPT, MSC, USAMRIID

COOPERATING UNITS *(if any)*

K. -I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 1.1 | .5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic mechanisms of phase variation in Coxiella burnetii, the etiological agent of Q fever, are not known. However, various strains with different virulence factors and lipopolysaccharide structure were studied to obtain possible molecular correlates of phase transition. A. Genetic heterogeneity. Chromosomal and plasmid DNA have been extracted from six isolates of C. burnetii. Restriction fragment length polymorphisms (RFLP) detected after Hae III digestions of DNA revealed four different patterns that distinguished American from the European isolates. RFLPs were also observed between the Nine Mile phase I and phase II prototype strains. At least one of the Hae III fragments visible in the pattern from Nine Mile phase I and missing in that from Nine Mile phase II could not be detected by DNA-DNA hybridization, and thus may have been deleted during the phase transition. Strains from two human endocarditis cases showed the greatest divergence. There were at least five fragments of unique mobility in the Hae III digestion pattern of DNA from the endocarditis isolates. Also, the plasmid obtained from these two isolates was two to three kilobases larger than the plasmid present in the other five isolates. B. Lipopolysaccharide (LPS). Phase variation in LPS structure from smooth (S) to rough (R) correlates with a shift from virulent (phase I) to avirulent (phase II) C. burnetii. The S and R LPSs were different in chemical composition and microheterogeneity. LPSs studied with the electron microscope were ribbon-like or they exhibited hexagonal lattice structures. The hexagonal lattice structures formed in vitro were due to the interaction of LPS II and the staining reagents rather than protein-LPS interactions. Significance: Detection of chromosomal and plasmid RFLPs and variation in LPS structure among strains of C. burnetii from various geographic locations and environmental sources will facilitate Q fever diagnosis and strain identification.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00183-07 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Properties of Coxiella burnetii (Q Fever) Vaccine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

J. C. Williams, PhD, Sr. Scientist, OSD, Group Leader, Rickettsial Diseases Laboratory, USAMRIID
E. H. Stephenson, DVM, PhD, COL, VC, Chief, Airborne Diseases Division, USAMRIID
C. E. Snyder, Jr., PhD, CPT, MSC, USAMRIID
G. H. Scott, PhD, USAMRIID
V. S. Sanchez-Carlo, PhD, NRC Post-Doctoral, USAMRIID
D. M. Waag, MS, PhD Candidate, OSD, NIAID

COOPERATING UNITS (if any)

K.-I. Amano, Hirosaki University School of Medicine, Hirosaki, Amori, Japan
F. T. Koster, Dept. of Medicine, University of New Mexico School of Med., Albu., NM
J. S. Goodwin, Dept. of Medicine; University of New Mexico School of Med.,Albu., NM

LAB/BRANCH

Office of the Scientific Director, NIAID, Bethesda, MD

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.3 | 3.3 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Candidate human vaccines against Coxiella burnetii, the etiologic agent of Q fever, have been studied in animals and in man in order to evaluate the humoral and cellular mechanisms of pathogenesis. A. mice. Fractions of phase I C. burnetii which induce mitogenic hyporesponsiveness and negative modulation of C57BL/10 ScN lymphocytes have been identified and partially purified. An infection model using C57BL/6J (resistant) and A/J (sensitive) mice was successful in demonstrating a 1,000-fold difference in the lethal dose 50 and a 7,000-fold greater sensitivity of the A/J mouse to the effects of immunosuppression. The concurrence of pathogenic and immunosuppressive events with infection or vaccination suggested that these responses were linked. However, studies with the immunological status of BALB/c sublines and congenic strains after either infection or vaccination have shown that gross pathologic responses can be genetically unlinked from the immunological unresponsiveness. Splenic lymphocytes of BALB/c mice were suppressed after infection or vaccination, but only infection induced hepato-splenomegaly. The splenic lymphocytes produce soluble lymphokines and suppressors during a 48-hour in vitro incubation with C. burnetii antigens. The cell types involved in the immunomodulation bind C. burnetii antigen, adhere to nylon wool, and are not completely inactivated by anti-theta and complement. B. Humans. Specific T-cell unresponsiveness is an important factor in persistent Q fever. Lymphocyte unresponsiveness to Coxiella antigen in patients with Q fever endocarditis was antigen-specific and was mediated, in part, by glass adherent suppressor cells. Prevention of protaglandin $E_2$ ($PGE_2$) by indomethacin completely reversed the Coxiella-induced suppression. Thus, elicitation of suppressors was antigen-specific and involved a T-Cell monocyte suppressor circuit. Significance. The objectives of this project are to define the genetic factors involved in susceptibility to infection and and phase I vaccine in the mouse model and to characterize the virulence factors of Coxiella which induce pathological reactions in humans.



PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00228-04 OSD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Flow Cytometric Analysis of Cell Membrane Antigens & Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

M.J. Waxdal, Senior Investigator OSD/NIAID
Ron Fico, CSC/DCRT

COOPERATING UNITS *(if any)*

Claudine Kieda and Michel Monsigny, Centre National de Recherche Scientifique, Orleans, France; Nathan Sharon, Weitzman Institute, Rehovoth, Israel

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 0.3 | 1.8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To establish, maintain and operate a state-of-the-art flow cytometry facility to serve the needs of NIAID's scientists. To analyze cellular differentiation markers, especially those which may be of value in disease diagnosis and staging cell-cell recognition, and intracellular signalling.



PHS 6040 (Rev. 1/84) GPO 904-917

LABORATORY OF BIOLOGY OF VIRUSES

1984 Annual Report

Table of Contents

# LABORATORY OF BIOLOGY OF VIRUSES

## National Institute of Allergy and Infectious Diseases

## SUMMARY - October 1, 1984 - September 30, 1985

The past several years have seen a significant change in the focus of work within the Laboratory of Biology of Viruses. Recombinant DNA technology and DNA sequencing techniques have permitted the examination of conceptionally new problems. For most of the small viruses, precise knowledge of virion structure has been available but recombinant methodology has allowed detailed analysis of regions of the larger viruses to be defined. Using data obtained by DNA sequencing, information on viral specified RNA can now be compared with the DNA template from which it was copied. This has provided an understanding of how simple genomes can generate multiple viral proteins by using alternate splice sites to form overlappping but unique messenger RNA molecules. The precise localization of the start sites for transcription of specific genes has permitted the identification of regions that lie upstream from the start sites which regulate the rate of transcription. The localization of these control regions has allowed investigators to enhance or suppress expression of specific genes by introducing site specific mutations into these control regions.

Regulatory mechanisms that function to control viral and cellular genes share a number of common properties. What is unique to the virus is that when it goes through a lytic cycle of growth, a single virus particle gives rise to 10,000 progeny virus particles and so strong selective evolutionary forces are operative on viruses. As a consequence, organization of genetic information in viruses will be unusually efficient and their regulatory structures anticipate what may ultimately occur with higher forms of life which evolve more slowly. The present annual report provides examples of this from studies with adenoviruses, adeno-associated virus and simian virus 40.

Laboratory of Biology of Viruses

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1983 - September 30, 1984

## HONORS AND AWARDS

Dr. Norman P. Salzman continued to serve on the Editorial Board of the Journal of Virology. He was re-appointed as Professorial Lecturer, Georgetown University School of Medicine and Dentistry for the 1986 academic year and was appointed to the Fogarty Scholars Selection Committee. He presented several invited lectures.

Dr. James Rose continued to serve as an Associate Editor of the journal, Virology (January, 1984).

## ADMINISTRATIVE CHANGES

Dr. Lois Salzman, a member of the Laboratory of Biology of Viruses since 1968, recently accepted an appointment as Assistant to the Scientific Director, NIAID.

Dr. Pierre May and Dr. Evelyne May of of the Institute de Recherches Scientifiques Sur le Cancer, Villejuif, France will be associated with the Laboratory of Biology of Viruses for a one year period starting September 1985. Dr. Pierre May is a Fogarty Scholar and Dr. Evelyne May is a Visiting Scientist.

Some of the highlights of this year's research efforts are detailed below.

STUDIES WITH PAPOVAVIRUSES (SV40 AND JC)

A. Regulation of Early Gene Expression: There is Competition between T-antigen and RNA Polymerase for a Shared Promoter Element

A set of nine mutants containing point mutations, and small deletions or insertions, were constructed in the early promoter region of SV40 to determine the role of the DNA sequences between the TATA box and the six upstream GC rich clusters in early transcription. The mutant templates were tested for *in vitro* transcription in HeLa cell extracts and *in vivo* in CV1 and COS cells using the chloramphenicol acetyl transferase gene (CAT) assay. Both *in vitro* and *in vivo* results show that the narrow region between nucleotide position (np) 38-41 is an important domain of the early promoter. Deletion and insertion mutations most strongly affect the level of transcription. While a four base pair deletion in the promoter region enhances the level of transcription four to six-fold *in vitro*, it causes a four-fold suppression of CAT gene expression in an *in vivo* assay. These opposite effects may result from changes in spacing under *in vitro* and *in vivo* conditions between two domains where transcription factors make simultaneous contact. Of the three T-antigen binding sites (I, II, III) sites I and II have already been shown to be involved in the autoregulation of the early transcription. Our mutational analyses demonstrate the role of site III, which partially overlaps with np 38-41, in the autoregulation of the SV40 early promoter. (Das and Salzman)

B. Regulation of Late Gene Expression: A "Surrogate" TATA Box and the Transcription Start Site May Serve as the Signal for the Start of Transcription.

The presence of a surrogate TATA box sequence located ca. 30 nucleotides upstream of the major late RNA state at nucleotide position (np) 325 (Brady *et al*., Cell 31:625-633, 1982) has been confirmed, and its structural speci-ficity has been determined by the generation of additional base substitution mutations at the *Kpn*I restriction site (np 294) in cloned simian virus 40 DNA. Two mutants generated new RNA initiation sites upstream of the np 325 start site and continued to utilize the authentic start site, but with decreased efficiency. The replacement of either one or both cytosines by thymines at np 298 and np 299 specifically enhanced *in vitro* transcription from the np 325 start site by 430 and 800%, respectively. This enhancement was due to con-version of the simian virus 40 late promoter present in the wild-type virus to a sequence that is similar to the TATA box present in the simian virus 40 early promoter. (Nandi and Salman)

C. GC Rich Promoter Sequences Can Specify the Start Site for Transcription

DNA sequences located within the simian virus 40 (SV40) G-C rich, 21 base pair repeats constitute an important transcriptional control element of the SV40 late promoter (Brady *et al*., Mol. Cell. Biol. 4:133-141, 1984). To gain further insight into the mechanism by which the SV40 G-C-rich repeats function, transcriptional properties of several recombinant DNAs have been compared. The results suggest that the SV40 GC-rich sequences can function

as independent RNA polymerase II transcriptional-control elements. In vitro competition studies demonstrated that sequences within the GC-rich, 21 base pair repeats, in the absence of either the SV40 early or late -25 transcriptional control signals or the major RNA initiation sites, efficiently competed for transcription factors required for SV40 early and late RNA synthesis. Transcription studies also demonstrated that in the absence of contiguous SV40 transcription control sequences, GC rich sequences stimulated initiation of transcription in a bidirectional manner, from proximally located sequences. The 21 base pair repeat region can also stimulate in vitro transcription from the heterologous adenovirus 2 major late promoter. (Mishoe, Brady and Salzman)

D. JC Virus Enhancer-Promoter that is Active in Human Brain Cells Shares Sequence Homology with Rat Brain Sequences

The enhancer region that contains two 98 base pair repeats was isolated and inserted into a plasmid containing the gene chloramphenicol acetyl trans-ferase (CAT) in different orientations and at different distances from the CAT gene. These plasmids were transfected into several cell types. CAT gene expression was only seen in fetal glial cells. This enhancer activity corre-lates well with the ability of JCV to grow in fetal glial cells. There is marked homology between the JCV 98 bp repeat and sequences present in precursor mRNA molecules that are unique to the brain. This suggests the presence of a brain specific transcription factor that recognizes both the JCV transcription regulatory region and the unique 82 nucleotide rat brain sequences. (Kenney, Natarajan, Salzman)

ADENO-ASSOCIATED VIRUSES (AAV)

A. Replication of the Defective Virus AAV Can Be Supported by Human Cytomegalovirus

Several early adenovirus (Ad) gene products are required for replication of defective parvoviruses (AAV; Janik et al., Proc. Natl. Acad. Sci. USA 78:1925-1929, 1981). Definition of specific helper functions of these factors would provide insight into biochemical details of AAV replication and, conversely, could help to determine their individual roles in the regulation of Ad macromolecular synthesis. Current studies are directed at determining the mechanism by which Ad VA RNA(s) and DNA-binding protein enhances translational expression of AAV mRNA species. It is clear that efficient translation requires the presence of both of these Ad factors. A similar analysis of specific herpes simplex virus (HSV) requirements for AAV replication is also being carried out, and we now have prepared an extensive library of subgenomic HSV clones.

A previous report concluded that human cytomegalovirus (HCMV) promoted synthesis of AAV capsid proteins, but did not allow for complete replication of infectious AAV (Blacklow et al., Proc. Soc. Exp. Biol. Med. 134:952, 1970). We have now established that HCMV is a fully competent helper virus for AAV replication as are both Herpes simplex viruses (Buller et al., J. Virol. 40: 241, 1981) and adenoviruses. Coinfection of AAV with HCMV strain Towne in human embryonic fibroblasts resulted in accumulation of AAV capsid antigen and production of infectious AAV with a lag of 24 h compared to AAV replication in AAV-adenovirus coinfections. In addition, HCMV and AAV were

synergistic in their cytopathic effects on cells, suggesting the possibility that AAV may play a role in the pathogenicity of HCMV infections.

We are currently studying the incidence of AAV in HCMV isolates obtained from patients to determine whether AAV may contribute to the pathogenicity of HCMV infections. We are also investigating early regulatory functions of HCMV replication which are required for the replication of AAV. (K.K.Wong, Sebring, Rose)

B. VA Genes Enhance AAV Protein

Following the initial discovery of the VA gene in Ad DNA (Rose *et al.*, Virology 27:571-579, 1965), its involvement in the virus replication process was uncertain until studies from our laboratory revealed that the VAI RNA gene was required for replication of defective parvoviruses (AAV; Janik *et al.*, Proc. Natl. Acad. Sci. USA 78:1925-1929, 1981). We have now shown that the VA gene products (relatively short RNA molecules, approximately 160 nucleotides in length) exert their enhancing effect at the level of translation and apparently do so in conjunction with a second early Ad gene product, the DNA-binding protein (DBP). In addition, we also have observed that DBP alone can modulate translational expression of specific Ad and AAV mRNAs. In more recent work, we have found that SV40 gene products do not substitute for all Ad DBP functions in restrictive monkey cells. (McPherson, Klessig and Rose)

C. Genetic Mapping of Sequences that Specify the Viral Structural Proteins

Our group previously demonstrated that the adeno-associated virus (AAV) contains three structural protein species: A, B, and C (90, 72 and 60 kilodaltons [kd], in the case of AAV2). In addition, we have detected four distinct subspecies of C and two of A. The three primary capsid proteins also have been shown to contain overlapping amino acid sequences (R. McPherson and J. Rose, J. Virol. 46:523-529, 1983). We have now shown that these proteins are encoded by a long open reading frame located in the right half of the genome. The coding capacity distal to the first ATG in this reading frame is only 503 amino acids (i.e., a protein about the size of C), but an open frame sequence devoid of ATG codons extends upstream for an additional 184 codons. Although the amino-terminus of the C capsid protein is blocked, partial amino acid sequence analyses of peptides from C have confirmed that it is encoded within the portion of the reading frame distal to the first ATG at nucleotide location (N) 2810). The amino-terminus of the B capsid protein is not blocked, and its sequence begins with alanine. The triplet encoding this alanine lies 64 codons upstream from the initiation site for C and is immediately preceded by the threonine codon, ACG, at N2615. This ACG codon lies in the most favorable sequence context for protein synthesis initiation. All three AAV2 capsid proteins are labeled *in vitro* with formyl-$^{35}$S]methionyl-tRNA$_f$, indicating that synthesis of each protein is initiated independently. Our data suggest that the N2615 ACG codon directs the methionyl-tRNA-dependent initiation of the AAV2 B capsid protein. Proteins B and C may be synthesized from the same mRNA species and their relative abundance determined by the efficiencies of their respective initiation codons.

Several putative non-structural polypeptides have been identified by hybridization selection and *in vitro* translation of AAV RNA. Additional

studies are now underway to map the genomic locations of these proteins and identify the specific mRNAs responsible for their production. (Becerra, Anderson, Rose)

D. Intermediates in AAV DNA Replication Intermediates Have Been Identified

The overall scheme of AAV DNA synthesis *in vivo* was first described in our laboratory (Straus *et al*., Proc. Natl. Acad. Sci. 73:742-746, 1976). Briefly, following coinfection of KB cells with AAV and a helper Ad, AAV DNA synthesis is initiated on single-stranded genomic templates by a self-priming mechanism. Subsequent elongation yields a unit length hairpin intermediate. A second round of self-primed synthesis displaces the 5'-ended arm of the hairpin and leads to either (i) displacement of a complete plus or minus progeny strand (by virtue of a processing/synthesis step at the closed end of the hairpin) or (ii) concatemeric molecules if closed end processing/syn-thesis does not occur. These latter molecules can be eventually processed to unit length templates which, in turn, may also yield progeny strands by new self-primed rounds of displacement synthesis. It has been suggested that a similar synthetic mechanism may be involved in the replication of cellular DNA. At present, the specific enzymatic and regulatory factors (both cellular and viral) that participate in AAV DNA synthesis are not clearly defined. To help identify and characterize these elements, we have utilized *in vitro* DNA synthesizing systems that generate AAV DNA replicating forms which correspond to those found *in vivo*. One of these systems consists of Ad-infected or Ad/AAV infected cytosol, uninfected nuclear extract and a DNA-protein template released from purified AAV virions. Analyses of the *in vitro* synthesized products reveal the presence of duplex unit-sized hairpin and non-hairpin molecules (replicative form [RF]DNA) and concatemeric structures. An initial replicating intermediate which precedes self-primed synthesis of duplex RF molecules has also been detected, and the putative processing endonuclease has been tentatively identified. This mode of replication differs from that catalyzed by *E. coli* DNA polymerase I and provides further insight into the self-priming mechanism for AAV DNA replication. In other studies, specific internal nicking of AAV concatemeric RF molecules has been demonstrated by a two-dimensional gel electrophoretic technique. (Ohi and Rose)

STUDIES WITH ADENOVIRUSES

A. Control Signals that regulate Expression of the $IVa_2$ Gene in Vitro.

The RNA initiation sites of the adenovirus $IVa_2$ and major late promoters (MLP) are separated by 210 base pairs and are transcribed from opposite DNA strands. We had previously shown that they contained overlapping promoter sequences [V. Natarajan *et al*., (1984) Proc. Natl. Acad. Sci. USA 81:6290-6294]. The transcription efficiencies of these two promoters were studied *in vitro* using templates of covalently closed circular DNAs that contained various deletion and point mutants. The distal control region of the $IVa_2$ promoter that is located at np (nucleotide position) -152 to -242 from the RNA initiation site consists of at least two domains. The first distal domain present between np -152 and -179 is necessary for efficient transcription of the $IVa_2$ promoter and it overlaps with sequences that have been shown to be necessary for efficient transcription of MLP. This region may serve as the entry site for the transcription machinery. The second distal domain consists of sequences

present between np-211 and -242. These sequences are contained at the 5' end in the MLP transcript. However, these sequences are not necessary for transcription of the MLP but they inhibit transcription from the $IVa_2$ promoter.

The 'TATA' box that is located at np -180 to -186 between these two domains has a critical role for efficient transcription of the MLP. A point mutation that reduces transcription from MLP by more than 80% stimulates transcription from $IVa_2$ promoter by 10-fold. This finding is consistent with the proposal that MLP and $IVa_2$ promoters share an entry site for transcription machinery and compete for its use. (Natarajan and Salzman)

B. Control Signals that Regulate Expression of the $IVa_2$ Gene in Vivo are Activated by the Adenovirus E1A Gene

The transcriptional control region of the adenovirus $IVa_2$ promoter was analyzed by cloning this promoter in front of a gene coding for bacterial chloramphenicol acetyl transferase (CATase) and estimating levels of CATase and $IVa_2$ promoter specific RNA synthesized after transfection. To produce detectable amounts of CATase with the $IVa_2$ promoter, an enhancer has to be present in cis. In the absence of enhancer sequences, the adenovirus E1A gene can not stimulate CATase synthesis. When cells were transfected with plasmids containing enhancer sequences and various $IVa_2$ mutant promoters upstream of the CAT gene, we observed that CATase activity was not reduced significantly even after deletion of all sequences upstream of the RNA initiation site.

Synthesis of $IVa_2$ specific RNA was dependent on plasmids containing an enhancer (SV40 72 bp repeat) that was present in cis. In the absence of enhancer sequences, co-transfection to provide the adenovirus E1A gene in trans also stimulated $IVa_2$ RNA synthesis. When HeLa cells were transfected with various deletion mutants with an enhancer in cis, it was seen that sequences -38 to -64 base pairs upstream of the RNA initiation site are necessary for efficient transcription. The E1A gene in trans and an enhancer in cis have an additive effect on RNA synthesis from both $IVa_2$ and major late promoters. (Natarajan and Salzman)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00290-04 LBV

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Deletion Mutants in Control Regions that Regulate SV40 Transcription

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Norman P. Salzman, Ph.D.

Others: Asit Nandi, Ph.D. Visiting Fellow LBV, NIAID

COOPERATING UNITS *(if any)*

Gokul Das, Ph.D., National Eye Institute, NIH, and Helena Mishoe, Ph.D., National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.7 | 1.3 | .4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In most eucaryotic genes, a common set of nucleotides has been found before the start site of RNA transcription. These nucleotides, referred to as either a TATA or Goldberg-Hogness box, are important determinants of transcription in vivo and in vitro. The control region for the late SV40 transcripts have been examined by site specific mutagenesis and by generation of deletion mutations. Mutations that enhance or suppress the transcriptional activity of a single start site have been identified and differ from the consensus Goldberg-Hogness box. When this upstream late transcription control region is converted to a sequence that is similar to the TATA box present in the SV40 early promoter, there is the strongest enhancement of late transcription in vitro. Spacing between a required GC rich domain and the TATA effects the level of early transcription suggesting that there is simultaneous contact in RNA polymerase II at these two sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00292-04 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Factors Required for Specific Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Norman P. Salzman, Ph.D. | Chief | LBV, NIAID |
| Others: | V. Natarajan, Ph.D. | Visiting Associate | LBV, NIAID |
| | M. B. Vasudevachari | Visiting Fellow | LBV, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL: | OTHER |
|---|---|---|
| 3.9 | 2.0 | 1.9 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro transcription systems are used to identify promoter signals and factors that control transcription in eucaryotic systems. These systems accurately initiate transcription from a wide variety of promoters. Under modified assay conditions, expression of the adenovirus $IVa_2$ gene is readily observed in vitro. This adeno gene, like the SV40 late genes, lacks a TATA box upstream from the 5' start site. By constructing a series of upstream deletion mutations, two upstream domains have been identified that control this gene. The distal domain only functions when covalently closed DNA is used as a template and contains within it two separate regions. One is believed to function as the entry site for RNA polymerase II while the second region inhibits transcription of the $IVa_2$ gene.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00293-03 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Characterization of Proteins Associated with the Parvovirus, KRV, and its DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lois A. Salzman, Ph.D. Research Chemist LBV, NIAID

Others: Dale Brown, Ph.D. Guest Worker LBV, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects XX ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

This project has been terminated.



PHS 6040 (Rev 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00294-04 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
PI: James A. Rose, M.D. Section Head LBV, NIAID

COOPERATING UNITS (if any)
(1) Richard McPherson, M.D., Department of Pathology, Georgetown University Hospital, Washington, D. C., (2) Daniel Klessig, University of Utah School of Medicine, Salt Lake City, Utah

LAB/BRANCH
Laboratory of Biology of Viruses

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Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
|---|---|---|
| .9 | .3 | .6 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Among objectives of these studies has been the application of physical, biochemical and biological techniques to characterize the structure and functions of certain segments (i.e., the inverted terminal repeat) and genes of adenovirus (Ad) DNA. In earlier studies, we first identified and characterized the VA RNA gene/transcript and inverted terminal repeats in Ad DNA. We have continued to investigate the specific regulatory functions of several early Ad genes, e.g., the VA and DNA-binding protein genes. Our results indicate that these latter genes are involved in the regulation of translation of certain viral mRNAs. Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis and DNA transfection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00295-04 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | James A. Rose, M.D. | Section Head | LBV, NIAID |
| Others: | Lalji Mishra, Ph.D. | Visiting Associate | LBV, NIAID |

COOPERATING UNITS (if any)
Richard McPherson, M.D., Dept. of Pathology, Georgetown Univ. Hosp., Wash., D. C., Leonard Rosenthal, Ph.D., Dept. of Microbiology, Georgetown Univ., Wash., D.C., John Hay, Ph.D., Dept. of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD

LAB/BRANCH
Laboratory of Biology of Viruses

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | .3 | .9 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects XX (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human parvovirus (AAV) genomes and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesviruses) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and continue to investigate their specific helper functions. Similar studies are in progress with herpes simplex viruses. In addition, we have now demonstrated that human cytomegalovirus is a competent helper for AAV multiplication, and we are attempting to map and characterize the required cytomegalovirus genes. Among methods used are specific immunofluorescence, cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses and DNA transfection of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00296-04 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


| | | | |
|---|---|---|---|
| PI: | James A. Rose, M.D. | Section Head | LBV, NIAID |
| Others: | Edwin Sebring, Ph.D. | Research Chemist | LBV, NIAID |
| | Patricia Becerra, Ph.D. | Visiting Associate | LBV, NIAID |


COOPERATING UNITS (if any)
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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.3 | 1.7 | .6 |

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The main objectives of these studies are (i) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (ii) to define the mechanism(s) by which the AAV proteins arise and (iii), to define specific functions of the AAV proteins. We have identified several AAV non-structural proteins which were previously undetected. At least one of these proteins is necessary for viral DNA replication. Post-translational processing does not account for production of any AAV structural proteins, although they share large proportions of sequences-in-common. It is now clear, however, that these proteins originate from independent in-frame initiations. The mechanism that regulates translation of AAV proteins is of fundamental interest and is now being investigated. Among methods used are affinity chromotography, gel electrophoresis, in vitro translation of viral RNA, electrophoretic and HPLC analyses of V8 protease and tryptic peptides and aminoterminal sequencing of purified polypeptides.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00297-04 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanism and Regulation of Adeno-associated Virus DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | James A. Rose, M.D. | Section Head | LBV, NIAID |
| Others: | Edwin Sebring, Ph.D. | Research Chemist | LBV, NIAID |
| | Seigo Ohi, Ph.D. | Visiting Associate | LBV, NIAID |
| | Kamehameha Wong, M.D. | Medical Staff Fellow | LBV, NIAID |

COOPERATING UNITS (if any)

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SECTION
Molecular Structure Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 3.7 | .8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects XX (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary objective of this project is to define biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) and adenovirus DNA replication in in vitro systems. We have now shown that replicating forms of AAV DNA can be generated in vitro utilizing either endogenous or exogenously added templates and cellular polymerase, and that de novo initiation of DNA synthesis can occur in vitro. Two potent inhibitors of AAV DNA synthesis have been purified from KB cells. Among methods used are differential centrifugation, ion exchange and affinity chromatography gel electrophoresis and isoelectric focusing.



PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00414-02

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

JC Virus - A Human Virus that Replicates Efficiently in Brain Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Norman P. Salzman, Ph.D.

| | | | |
|---|---|---|---|
| Others: | Shannon Kenney, M.D. | Medical Staff Fellow | LBV, NIAID |
| | V. Natarajan, Ph.D. | Visiting Associate | LBV, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
|---|---|---|
| 1.5 | 1.1 | .4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A human papovavirus, JCV, is the etiologic agent of the fatal demyelinating disease, progressive multifocal leukoencephalopathy. The JCV 98-base-pair tandem repeats, located to the late side of the viral replication origin, were shown to be a transcriptional regulatory element with enhancer-like activity in human fetal glial cells. These tandem repeats share significant homology with the 82-nucleotide rat brain-specific identifier RNA sequence.



PHS 6040 (Rev 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 AI 00446-01 LBV

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)
RNA Polymerase II

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Norman P. Salzman, Ph.D. | Chief | LBV, NIAID |
| Others: | Gerald Selzer, Ph.D. | Senior Staff Fellow | LBV, NIAID |
| | Rajesh Singh, Ph.D. | Visiting Fellow | LBV, NIAID |
| | Feng-Sheng Xu, Ph.D. | Visiting Fellow | LBV, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Biology of Viruses

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS | PROFESSIONAL | OTHER |
|---|---|---|
| 5.3 | 2.4 | 2.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

Six hybridomas that make antibody against RNA polymerase II have been obtained and cloned. They are presently being characterized to determine their subunit specificity.



PHS 6040 (Rev. 1/84)

GPO 904-917

LABORATORY OF CLINICAL INVESTIGATION
1985 ANNUAL REPORT
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Laboratory of Clinical Investigation

Laboratory of Clinical Investigation

## SUMMARY OF PROGRAM

Laboratory of Clinical Investigation
October 1, 1984 to September 30, 1985

Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

## INTRODUCTION

The year ending October 1985, has continued to be a very productive one for the Laboratory of Clinical Investigation. This has been a year of stability in our programs which are now widely recognized. Both senior and junior members of the staff are highly sought after as participants in international meetings, and as Board Members of the major societies that are associated with their respected areas of research or clinical practice. The program continues to be divided into three major program areas -- Allergic Disease, Infectious Disease, and Clinical Immunology. The Allergic Disease Program is headed by Dr. Michael Kaliner, working in conjunction with Dr. Dean Metcalfe. The Infectious Disease Program consists of sections led by Drs. Gallin, Straus, Bennett, and Quinn, with Dr. Eric Ottesen occupying a section jointly in the Laboratory of Parasitic Disease and the Laboratory of Clinical Investigation. Other members of the group include Drs. Kwon Chung, Nath, Seligmann, and Ostrove. The Clinical Immunology Program consists of the sections of Drs. Frank and Strober working with Drs. Joiner and James.

These programs encompass a large number of areas under the general heading of host defense. There is considerable opportunity for flexibility in the research program and considerable opportunity for collaboration and cooperation between the various sections. We continue to cooperate closely with members of the Laboratory of Immunoregulation under Dr. Anthony Fauci.

### Clinical Immunology Program

### Mucosal Immunity Section

Dr. Warren Strober and Dr. Stephen James continue to study the factors that control immunity as expressed and developed across mucosal barriers. They are developing methods for study of immunologic gastrointestinal disease. As part of these studies they have continued to examine the factors that control IgA immunoglobulin synthesis. This is the principle immunoglobulin that protects the mucosal surface of the GI tract. The group is beginning to understand what controls the differentiation of B cells into IgA bearing and secreting cells in the gut. The production of IgA involves the switching of B cells from the production of other immunoglobulin classes to the production of IgA. The group originally showed that Peyer's Patch T cells are important in such switching. They have developed T cell hybridomas which are capable of mediating this effect. More recently they have shown that antigenic stimulation as occurs with bacterial lipopolysaccharide may also be important in the switch phenomena. In examination of immunoglobulin synthesis and secretion, they now can find message for the various immunoglobulin classes and identify such message. Their recent results show that T cells derive from Peyer's patches can induce changes in Ig class expression in a pre-B cell line, suggesting

immunoglobulin switch mechanisms are operative or that there is increased transcription of pre-existing IgG messenger RNA.

Strober and Kotani have also been studying the autologous mixed lymphocyte reaction, a proliferative response to T cells induced by contact with autologous B cells and macrophages. This is a general phenomena and its' relationship to autoimmunity is being explored in a number of laboratories. These workers have found that autoreactive T cells develop in culture when it is stimulated by antigen repeatedly and such cells can be isolated and identified. Clones of such cells have now been prepared and can be studied. Some of these autoreactive clones have helper function as well and can increase production of immunoglobulin by B cells. The data that these workers have accumulated suggest that autoreactive T cells have a dual regulatory capability which is differentially expressed by the mode of activation. When stimulated by major histocompatability antigens present on unactivated B cells, they provide helper activity. When stimulated by these same antigens on activated B cells, they induce active expression. The mechanisms for these effects are under study at the present time.

Studies have also been carried out by Drs. James and Strober in collaboration with Dr. Jones of the Liver Disease Section, NIADDK, of patients with primary biliary cirrhosis. The thrust of these studies is to determine various lymphocyte subset activity in this disease. Their preliminary findings thus far suggest that only a small subset of activated B cells may contribute to the autoimmune process, including hyper-IgM synthesis and immune complex formation. They also showed that patients with primary biliary cirrhosis have deficient natural killer cell activity in their peripheral blood. They are currently studying this phenomena in an attempt to further understand the basis of this disease. One interesting finding is that a family with IgA deficiency has members with primary biliary cirrhosis, proving that IgA is not required for the development of this disease.

The group also is studying immunoregulatory defects in inflammatory bowel disease. Emphasis of the studies is on defining subsets of lymphocytes abnormal in these patients. For these studies lymphocytes are obtained from the periphery but also from bowel specimens taken at the time of resection. In peripheral blood, patients with Chroen's Disease have a similar proportion of lymphocytes of the suppressor/helper types as that noted in normals, as well as a normal number of cytolytic and effector cells. They find distinctly different proportions of cells in the intestinal lamina propria than those in the peripheral blood. Thus far they have not identified a specific subset abnormality in this disease but are learning a great deal about the normal physiology and pathophysiology of intestinal immunity. This has led Dr. James to further studies of the phenotypes and functions of intestinal lamina propria lymphocytes in nonhuman primates. Methods have been developed to identify the various subsets of lymphocytes in these animals. Moreover it has been possible to produce lymphogranuloma venerium in the GI mucosa of these animals. Induction of disease has led to major changes in the lymphocyte populations present. The study of this model continues.

The Clinical Immunology Section has continued to focus most of its' efforts on the role of complement in the Host Defense process. Two major areas have continued to be studied during the past year. The role of complement in the

lysis of gram negative organisms has continued to be studied by Drs. Joiner, Frank and their colleagues. This has led to increased attention to the function of late components of complement, not only in bacterial killing but also in autoimmune diseases in man. Drs. Brown, Frank, Gaither and Fries have continued to study various aspects of opsonization. Drs. Hammer and Tenner have continued studies of complement purification, biosynthesis and control. In addition, Dr. Joiner has carried out studies of the role of complement in control of parasitic diseases. As part of the studies of lysis of gram negative organism it became important to develop antibodies to a new antigen expressed on the complex of proteins of C5,6,7,8 and 9 when they joined together to form a membrane attack complex. These proteins form a cylinder-like structure which inserts into the surface of a cell or bacterium to destroy the integrity of that cell. New antigens are expressed by the complex and a sensitive ELISA assay was developed to detect that neoantigen. Of great importance is the fact that it is possible to use this ELISA assay to detect the neoantigen in body fluids, such as blood and spinal fluid. Initial studies have shown that 13 of 14 patients with the Guillian Barrè Syndrome have high levels of the C5-9 neoantigen in their spinal fluid at the time of disease, while it was only rarely found in normal individuals. Similarly, a large proportion of patients with Multiple Sclerosis have this material in their spinal fluid. The antibody developed can be used to detect this neoantigen in tissues as well and is currently being used in studies of tissues from patients with rheumatoid arthritis, osteo arthritis, and autoimmune and dermatologic disease.

These studies have also led to important new findings on the role of complement in the killing of gram negative bacteria. It was shown for one bacterial type that all of the late components were required for killing, unlike the situation with red cells. Moreover, it was possible to prove the five through nine complex must contain more than three C9 molecules per C7 in the complex for killing to occur. The role of antibody in directing the deposition of the five through nine complex has also been studied in detail. It has been possible to prepare monoclonal antibodies against specific cell surface proteins on gonococci that have different properties in their ability to lyse the gonococcus. This finding indicates that the antibody itself is highly stereospecific in directing the deposition of the C5b-9 complex. The epitope recognized by the antibody will determine the site of deposition of the C5b-9 complex and this in tern determines whether lysis will follow. This marked difference in the killing activity of the antibody is present even though equal amounts of complement are deposited on the bacterial surface with lytic and nonlytic types of antibody. The clinical relevance of this comes from the finding that antigonococcal "blocking" antibody, which blocks the activity of antigonococcal "bactericidal" antibody, and which occurs in certain individuals which may be at greater risk for gonococcal dissemination, does not decrease the amount of complement bound to the bacterial surface. Blocking antibody actually increases the amount of complement bound. The blocking antibody does compete for binding sites with the bactericidal antibody. In other studies it was shown that up to 1/5 of the C3 deposited on bacteria sensitized with bactericidal antibody and incubated in serum actually binds to antibody itself. In vitro production of such C3 antibody complexes has shown that it is far more bactericidal activity than the IgG antibody itself. Dr. Fries has studied the properties of-C3 IgG complexes in greater detail. C3 shows preferential binding for the heavy chain of IgG. In this site on the heavy chain, it is

relatively protected from the action of the inactivator proteins β1H and C3 inactivator. In this location it thus continues to function as active C3b. It is much more bactericidal than normal IgG. Other properties of this complex are being studied at present but it is of significance that this complex occurs in patients with various autoimmune diseases.

Other studies have examined on the process of opsonization and phagocytosis. Dr. Brown's group has developed an assay which allows one to study the binding of bacteria to phagocytic cell surfaces and to determine which bacteria are still bound to the surface of the phagocyte and which have been internalized. Using this type of approach it has been possible to show that the major receptor responsible for internalization of serum incubated pneumococci is the C3b receptor, even though multiple C3 products are found on the bacterial surface. They have also shown that laminin enhances phagocytosis of cells coated with the complement fragment C4b, iC3b and antibody alone. This is an extracellular matrix protein and may plan an important role in the regulation of phagocytic cell activity in the tissues. Studies of this group have examined the interaction of Clq and laminin. It has been shown that Clq tends to bind to laminin and the site on laminin molecule which binds Clq has been visualized by electron microscopy. Since laminin is present in basement membranes it may bind Clq to set of the autoimmune process under certain circumstances.

Frank, O'Shea and their colleagues have continued to study C3b and iC3b receptor activity on the surface of phagocytic cells. They showed that a variety of cell activators induce upregulation of the complement receptors. These include lymphocyte products as well as C5a and various chemotactic factors. They also showed that intracellular pools of latent receptor exist and were able to isolate specific granules from neutrophils and show that they contain C3bi receptors. The showed that phorbol esters induce the internalization of C3b receptors on neutrophils. However, although the receptors are internalized, the cells maintained heightened phagocytic activity for test particles coated with C3b, suggesting a dissociation between receptor number and receptor function. These authors also showed that phorbol esters cause an association of the C3b receptor with the cellular cytoskeleton, presumably facilitating both endocytosis and phagocytosis. They have proposed that the physiologic activation of CR1 (the C3b receptor) may occur via polyphosphoinositide metabolism. Moreover, they have clearly dissociated endocytosis per se and phagocytosis with certain agents like A23187 which inhibit phagocytosis but augment endocytosis via the C3b receptor.

Gaither and her colleagues have studied neutrophils from patients with chronic granulomatous disease and find that they have greatly increased phagocytosis and phagocytic activity when compared to normal cells. Further studies have now shown that this is true with phagocytes from a patient with myeloperoxidase deficiency. Moreover, normal phagocytes can be induced to behave like CGD phagocytes by treatment with sodium azide, which increases binding and phagocytosis by these cells enormously. It is proposed that this is a normal regulatory mechanism which controls the activity of phagocytes, which is deranged in individuals without normal oxidative function. Interestingly these workers have shown that the abnormal phagocytes from patients with these two diseases can be induced to perform normally by the addition of an exogenous $H_2O_2$ generating system, as supplied by glucose and glucose oxidase.

The receptor responsible for the phagocytosis of C3d coated erythrocytes has been studied in detail and shown to be the C3bi receptor. During the year, new techniques have been completed for the purification of large amounts of C5a and C3a. In addition, studies continue on the purification and function of the C1 inhibitor. It has been shown that C4 inhibits the activity of the C1 inhibitor in dissociating the C1 molecule on a cell. New assays have been developed which allow one to examine the functional activity of the C1 inhibitor in clinical specimens and a new assay has been developed which allows one to separate unactivated C1 from activated C1. This has presented enormous technical problems in the past (Tenner). The study of C1q synthesis has also continued with questions being asked about the mechanism of synthesis, the molecular basis for the disease in C1q deficient individuals, and the role of receptors for C1q on phagocytic cells in the function of these phagocytic cells. Functional studies with members of the Laboratory of Immunoregulation suggest that C1q may affect the differentiation of large lymphocytes into immunoglobulin secreting cells. In studies with collaborators at Johns Hopkins, it was shown by Hammer and his colleagues that antibody and complement cause a marked increase in arachidonic acid metabolism and the membranes of cells so treated. A complement inhibitor derived from *Aspergillus fumigatus* has also been studied in collaboration with members of the Clinical Mycology Section.

Finally, studies by Dr. Joiner in collaboration with Dr. Sher of the Laboratory of Parasitic Disease have defined the particular protein that binds C3 on epimastigotes during incubation in serum and the function of this protein. A 72,000 dalton glycoprotein was found to be the preferential C3 receptor. It was shown that 3/4 of the C3 on epimastigotes following 60 minute incubation in serum was present as C3b and the remainder was present as the inactivated product iC3b. However, on the resistance metacyclic trypomastigotes 85-90% of the C3 was present as the inactive product. It was shown that the location of C3 on the cells controlled the activity of the inactivating proteins H and I as well as the alternative pathway activating protein factor B. It would appear that the control of alternative pathway activation on these forms is exerted at the level of B binding primarily. It is planned to perform extensive study on the nature of the binding of complement to a variety of parasites of different types.

## Infectious Disease Program

### Bacterial Diseases Section

Several aspects of phagocyte biology have been studied using neutrophils (PMN) and monocytes. Investigation of the subcellular location of fmet-leu-phe, C3bi as well as for cytochrome b suggests these constituents are packaged in a common compartment similar to specific granules. In related studies a series of monoclonal anitbodies against the inside of the neutrophil plasma membrane have been developed with the goal of identifying specific secretory granule attachment sites. Studies in exudate PMN have revealed greater than five-fold more fmet-leu-phe receptors than blood PMN, presumably related to receptors mobilized from the intracellular pool.

In studies extending earlier work on neutrophil heterogeneity a monoclonal antibody, 31D8, that binds to a subpopulation of PMN was studied in

chronic myelogenous leukemia (CML). The data indicate there are two groups of CML patients; those with 31D8 bright or dull PMN. It is not clear whether or not the CML heterogeneity reflects differences in clonal proliferation. Unexpectedly absence of the 31D8 antigen appeared predictive of progression of CML to the accelerated phase.

Studies of IgE turnover in the Hyperimmunoglobulin E-recurrent infection (Job's) syndrome have revealed decreased clearance of IgE in subjects with elevated IgE. This has implications for numerous diseases in which IgE is elevated.

In other studies gamma interferon was shown to be a chemoattractant for PMN and monocytes and this may relate to gamma interferon's ability to induce macrophage giant cell formation.

The chemotactic peptide fmet-leu-phe and the $Ca^{2+}$-ionophore A23187, stimulate PMN tubulin tyrosinolation that is dependent on the presence of extracellular $Ca^{2+}$ and the activation of NADPH-oxidase mediated oxidative burst. Isolated PMN cytoplasts (CP) and karyogranuloplasts (KGP) fail to respond to either stimuli, indicating the requirement of an intact functional PMN for the modulation of PMN tubulin tyrosinolation. Unlike the specific stimulation of tubulin tyrosinolation in fmet-leu-phe or A23187-stimulated PMN, an intriguing phenomenon of tyrosine incorporation into multiple proteins was observed in PMA-activated PMN, that is dependent on the pathway for NADPH-oxidase activation and independent of protein synthesis. The reaction is inhibited by a variety of reducing agents and intracellular scavengers of oxygen radicals. PMA-activated PMN from patients with chronic granulomatous disease failed to exhibit this phenomenon, but activated PMN from a myeloperoxidase deficient patients incorporated the tyrosine. PMA-activation of PMN causes a two-fold increase in the generation of protein carbonyl derivatives, which is potentiated in the presence of labeled tyrosine. Reverse phase HPLC analysis of radiolabeled samples indicate the presence of radioactivity in multiple peaks with distribution throughout the protein (peptide) fractionation range. SDS-urea gel patterns also reveal similar results. The PMA-induced incorporation of tyrosine is highly exaggerated in KGP and also appears to be quite specific for tyrosine as other amino acids like phenylalanine, leucine, histidine or methionine, fail to incorporate. The biochemical mechanism and the functional role of this intriguing reaction remains to be elucidated.

To investigate mechanisms of phagocytic cell activation methods were developed to measure intracellular calcium (fura2 & indol) and membrane potential independent of mitochondrial potential (oxonol dyes). A pool of intracellular calcium regulates neutrophil function, a calcium signal is sufficient to stimulate superoxide and secretion, and there is selective inhibition of calcium dependent activation by PMA, possibly through protein kinase c mediated phosphorylation. Protein kinase was studied causing the activator di-C8 (diacylglycerol analog). di-C8 stimulates superoxide and secretion like PMA but low doses cause transient responses resembling chemoattractant effects.

The activation of mast cells and lymphocytes was investigated. Both calcium dependent and calcium independent activation mechanisms were found in mast cells. A calcium dependent membrane potential depolarization was elicited by IgE binding. These changes reflect a large calcium efflux and its role in

activation is now being defined using a series of cell lines with specific biochemical and functional defects. The studies with lymphocytes indicate that both T and B cells display a calcium dependent activated potassium flux which is sensitive to manipulation in cold or minus calcium media. Studies with T cells and lines transvected with the T cell receptor indicate these cells are activated mitogentically and functional display a rise in calcium when the T cell receptor is crosslinked by antibody.

## Clinical Mycology Section

*Aspergillus fumigatus* galactomannan was found by ELISA or RIA in urine from 7 of 8 patients with invasive aspergillosis caused by that species but not in urine from controls or from 2 patients infected with *A. flavus*. The assay appears useful in diagnosis of this potentially fatal infection.

*Aspergillus fumigatus* was found to produce an extracellular substance which inhibits the ability of normal human serum to opsonize fungal cells for ingestion by human monocytes. The substance impairs the alternative pathway of the complement cascade, causing reduced deposition of C3b on the fungal surface.

A rapid enzymatic assay for the antifungal drug, flucytosine, was described. Creatinine iminohydrolase (EC 3.5.4.21) was used to quantitatively remove the amino group from flucytosine. Ammonia was then measured colorimetrically.

Otherwise normal patients who are cured of cryptococcosis are specifically tolerant to immunization with cryptococcal polysaccharide. Recent work has shown that immunization of volunteers but not cured patients leads to circulating B cells with surface anticryptococcal antibody, indicating that tolerance in these patients occurs at an earlier stage of the humoral response.

Immunoglobulin allotypes Gm 1,2,3,17;23,5,6,13,12, Km1 and Km3 did not correlate with immune response of normal volunteers to cryptococcal or pneumococcal polysaccharide.

Other topics of current studies include:(1) characterization of a new fungal pathogen isolated from a granulomatous lesion of olecranon bursa;(2) Pathogenesis and virulence factor of *Candida albicans*: (3) biochemical genetics of resistance to 5-fluorocytosine (5-FC) in *C. albicans*; (4) development of a parasexual genetic system for *Torulopsis glabrata*. An isolate of *Anthopsis deltiodea* was found to be the cause of an olecranon brusitis in a man. *A. deltoidea* has never been reported previously from clinical specimens. *A. deltoidea* produced dematiaceous hyphae in tissue in the center of the necrotic debris. The relationship between extracellular proteinase and virulence for mice in *Candida albicans* was studied using an isogenic set of proteinase - producing parent (C9), a proteinase - deficient mutant (C9M1) derived from the parent by nitrouse acid treatment and a spontaneous revertant (C9M1M) obtained by mouse passage of C9M1. The isolate C9 produced a high level of proteinase *in vitro* and caused 100% fatal infection within 21 days. The mutant produced no detectable enzyme *in vitro* and all mice survived until day 22. Only 30% of the mice infected with C9M1 died between day 23 and 30. The isolates recovered from the dead mice were found to be proteinase sufficient. The C9M1M produced proteinase *in vitro* at 44% of the level of the wild type and induced fatal

infection in 90% of the mice within 30 days indicating that proteinase activity is one of the factors associated with the virulence of C. albicans. Resistance to 5-flucytosine in C. albicans results form a defect in UMP pyrophosphorylase activity (fcyl) or from a defect in cytosine deaminase activity (fcy2). Kwon Chung and associates have demonstrated complementation in fcyl FCY2 x FCY1 fcy2 crosses. A parasexual genetic system in Torulopsis glabrata was developed by spheroplast fusion between two haploid strains carrying different nutritional markers.

## Medical Virology Section

The pathogenesis, immunology, natural history and therapy of human herpesvirus infections are being investigated. Immunocompetent and immunodeficient patients, including those with AIDS, who possess a wide range of herpesvirus infections have been identified and studied. The major emphases has been on frequently recurring genital herpes simplex virus infections and on chronic Epstein-Barr virus infection. During the past year the group has completed a large longitudinal study of symptoms and virus shedding patterns in patients with frequently recurring genital herpes before, during and after suppressive acyclovir therapy. As part of that study they have identified and quantitated shedding of virus in the absence of symptoms. They observed and studied an episode of transmission of genital herpes associated with an episode of asymptomatic shedding. In terms of chronic Epstein-Barr virus infection, they have continued long term observation of a series of patients with a chronic fatiguing illness associated with elevated Epstein-Barr virus antibody titers. They initiated a placebo controlled trial of intravenous and high dose oral acyclovir therapy for patients with that disorder. During the coming year they plan to initiate another study of asymptomatic shedding in patients with genital herpes and to complete current placebo controlled acyclovir trial for chronic EBV infections.

The major emphasis on the study of the molecular biology and latency of varicella-zoster virus (VZV) has continued. Straus and colleagues are performing finer endonuclease mapping of varicella zoster virus DNA strains as well as completing a preliminary map of 58 viral encoded transcripts. They are using an extensive library of VZV DNA recombinants to map various VZV encoded gene products. Using marker rescue techniques they are attempting to map the genetic loci associated with VZV resistance to antiviral drugs. By hybrid selection and in vitro translation of viral RNAs and immunoprecipitation with polyclonal and monoclonal antibodies we are identifying and mapping major viral proteins. By tranasformation of thymidine kinase deficient mouse L cells they have identified and mapped the gene responsible for the VZV pyrimidine kinase enzyme. During the coming year the group plans to initiate in situ hybridization studies of human tissues for latent VZV DNA and RNA sequences.

## Thomas Quinn, John's Hopkins Affiliate
## Clinical Immunology Section

Dr. Quinn and his colleagues continue to play a major role in the development of the Institute's expertise in the Acquired Immuno Deficiency Syndrome both in this country as in Africa. Dr. Quinn has taken considerable time to help with the development of epidemiologic, virologic, and immunologic studies in Zaire and other places in Africa. Working with Drs. Francis and Fauci, they have

identified 400 cases of AIDS in Zaire over the last eight months and have shown that the male to female ratio is one to one. In this population the disease is predominantly transmitted heterosexually. Moreover, unlike in this country, household contacts have a much higher incidence of infection as shown by antibody studies. They have established an ELISA test for detection of antibody to the AIDS virus and have shown that it has a high degree of sensitivity and specificity. Studies of this important group are ongoing. Like patients in this country seropositivity to HTLVIII correlated with marked depression of T4 lymphocytes and anergy. In studies performed in this country of *in vivo* RES function in AIDS patients, it was shown that 11 of 15 patients with AIDS and 2 of 9 with AIDS related complex had prolonged Fc specific clearance rates as compared to control. C3b clearance rates were markedly abnormal in seven AIDS patients when compared to control. These patients cleared C3b coated cells normally but did not show the normal degree of phagocytosis of cleared cells seen in controls. The patients processed the C3b and released the cells back into the circulation suggesting that they have a major phagocytic defect. Similarly patients were shown to have a defect in antibody directed cell mediated cytotoxicity against chicken red blood cells coated with antibody. These studies continue. In addition the group has established a program for study of Chlamydia trachomatis infection. This is the most commonly transmitted sexually transmitted bacterial pathogen in the United States. Infection in pregnant women was found to be positively associated with prematurity of the infants and post abortion endonetritis. A new assay was developed to develop infection via an in situ DNA hybridization technique and a model was developed for studying this disease in primates. This model is being followed with members of the Mucosal Immunity Section under Dr. Strober, and promises to be an important addition to our understanding of host resistance across mucosal surfaces.

## Clinical Parasitology Section

The goal of these studies is to increase understanding of the pathogenesis, and to improve diagnostic and therapeutic measures for parasitic diseases.

A method of purifying bovine cryptosporidial oocysts for use as an antigen in an ELISA test was developed. IgM and IgG antibody responses were demonstrated in immunocompetent as well as in AIDS patients, with indications the *Cryptosporidium* is a common human infection. The criteria and specificity for an immediated hypersensitivity skin test for strongyloidiasis have been established. For immunodiagnosis of filarial infections efforts to improve the sensitivity assays for circulating filarial antigens are continuing.

Therapeutic trials underway include a double-blind prophylactic trial of DEC against acquisition of *Loa loa* infection in Peace Corps volunteers in Africa, treatment of cerebral cysticercosis with proziquantel, treatment of strongloidosis with Ivermectin, and use of ivermectin to treat lymphatic filariasis in India. Recombinant gamma interferon was found to show limited effects in treating anergic cutaneous leishmaniasis.

The relationship of the immune response to immunopathology in several types of helminthic and protozoan infection is being examined. The antigen-specific T-cell suppression in filariasis and leishmaniasis was demonstrated to be at

the level of lymphokine (IL-2 and gamma-interferon) production. The relationship between IgE and $IgG_4$ generating and inhibiting allergic responses to parasites was aided by the use of anti-subclass monoclonal antibodies. Broncho-pulmonary lavage revealed persisting alveolitis in tropical pulmonary eosinophilia patients after therapy, suggesting that more intensive treatment may be necessary.

*Giardia* isolates from various sources are being characterized as to their DNA composision, surface antigens and biologic behavior. Several isolates will be used for human volunteer infections.

## Allergic Diseases Program

### Allergic Diseases Section

Dr. Kaliner's group continues to study immediate hypersensitivity with the focus on human and animal models of allergic responses, mechanisms of mediator action and pharmacologic approaches to disease.

Employing monoclonal antibodies directed at cyclic GMP, the pattern of cells in guinea pig lung responding to histamine stimulation has been identified. Histamine causes all cells to increase their cytoplasmic cyclic GMP with an increased concentration near the nuclear membrane. In mouse lung, a population of dendritic cells has been identified which is found in the mucous membrane and is a potent antigen presenting cell population. Ketotifen has been found to prevent histamine release from mast cells in patients with physical urticarias. Histamine levels in plasma are diagnostic of systemic mastocytosis if consistently elevated. The mechanism for progesterone-related anaphylaxis has been examined and remains unclear while a second progesterone-sensitive subject with anaphylaxis responded to LHRH analogue therapy. Microvascular permeability in skin is increased by histamine, serotonin, and bradykinin but does not appear to contribute to the edema seen in late phase responses. Plasma histamine from patients in gram negative sepsis and shock is reduced below normal.

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to products generated by pulmonary macrophages and peripheral mononuclear cells with increased mucous glycoprotein secretion. The macrophage and mononuclear derived secretagogues are collectively being called macrophage/mononuclear cell derived mucus secretgogues (MMS).

Activation of complement leads to anaphylatoxin generation. Current studies indicate that anaphylatoxins may be formed in pulmonary inflammatory processes. Therefore, the effects of human C3a upon mucus release were examined. C3a (and C5a) cause a dose-related stimulation of mucus secretion, maximal at 1-4 hours, apparently not requiring mast cell activation and not reproduced by C3a des arg. Thus, complement derived anaphylatoxins may also participate in mucus secretion.

Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipomodulin generation and MGP production.

Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identity of the mucus secretagogue is under study.

Mast cells are the cellular nidus of allergic diseases and the cell responsible for disease in urticaria pigmentosa and systemic mastocytosis. One project of the section is designed to ablate mast cells by attaching cytotoxic agents to IgE or antigen and selectively introducing the toxic product into mast cells. IgE linked to ricin kills RBL cells spontaneously and after crosslinking the IgE with anti-IgE. IgE linked to ricin's A chain kills only in the presence of monensin, a carboxylic ionophore. Therefore, IgE-linked immunotoxins are a new and possibly useful way to ablate mast cells.

Allergic rhinitis is the most common chronic condition suffered by Americans, with about 7% of the population involved. Until recently, few studies of nasal physiology were being performed in this country. We are examining the effect of mediators and anaphylaxis on nasal blood flow and protein secretion by comparing atopic humans to normal controls. Nasal blood flow is not affected by methacholine but is reduced by alpha adrenergic agonists. Protein secretion is increased by methacholine and histamine in all subjects, but atopics are far more reactive than control populations. We are currently studying antigen challenge in these same populations.

## Mast Cell Physiology Section

The Laboratory under Dr. Metcalfe has continued to develop it's expertise in two major areas, mastocytosis and food hypersensitivity. They continue to be interested in proteoglycan chemistry as well.

A comparison of lung mast cells with gastrointestinal mast cells from the same monkey reveals both similarities and differences. Both populations of cells degranulate to anti-human IgE, but not to compound 48/80. Degranulation induced by anti-IgE in both mast cell populations is inhibited by theophylline and quercetin. However, in comparison to gastrointestinal mast cells, lung mast cells stain more readily, have a higher histamine content, and release more mediators upon stimulation, confirming mast cell heterogeneity and the need to characterize mast cell populations in higher animals.

Cultured mouse mucosal mast cells degranulate upon exposure to N-acetyl cysteine, a mucolytic agent. Mucosal mast cell degranulation is inhibited by sulphasalazine. Aspartame has little or no direct effects on mast cells and basophils.

Patients with a history of immediate adverse reactions to foods and whose symptoms are reproduced on challenge, are atopic, have multiple positive skin tests to foods and inhalants, have a positive skin test to the food in question, and by history are those with the most severe reactions. Twenty-five patients with idiopathic anaphylaxis, and eight with systemic mastocytosis have

been challenged with sulfites. No clinical reactions were observed, although plasma histamines were elevated following challenge. One severe reactions to sulfites was observed in an asthmatic.

Two forms of mastocytosis have been documented. Ninety percent of our patients have disease presenting initially as urticaria pigmentosa and which slowly progresses over decades. A second rapidly progressive form of mastocytosis presents with lymphodenopathy, peripheral eosinophilia, an elevated sedimentation rate, and an elevated alkaline phosphatase. One patient with this disease, which we have termed lymphadenopathic mastocytosis with eosinophilia, has had an initial response to a combination of cytoxan, vincristine, and prednisone. In a related observation, patients with malignancy and mastocytosis are more likely to have oligoclonal immunoglobulin bands on agarose gel electrophoresis, aiding in diagnosis.

Patients with systemic mastocytosis have elevated plasma histamine levels (approximately 2000 pg/ml). Patients with urticaria pigmentosa have slightly elevated plasma histamines, while patients with idiopathic anaphylaxis have normal histamines (approximately 270 pg/ml).

Systemic mastocytosis may be complicated by an increase in basal acid output, and by maladsorption. Such findings are highly variable, but tend to occur in patients with severe generalized disease.

The histamine content increases in cultures of human bone marrow in association with the appearance of poorly defined granulated cells which die out after approximately 6 weeks. Lectin-stimulated human peripheral mononuclear cells produce a factor which stimulates the growth of cultured, IL-3 dependent, mouse mast cells. Fibroblasts and endothelial cells both are capable of the phagocytosis and degradation of mast cell granules as demonstrated by microscopy and by the use of radiolabeled mast cell granules. Mast cell granules rapidly and selectively degrade extracellular fibronectin. While this degradation is due to chymase, mast cell granules are particularly efficient at cleaving fibronectin by virtue of their heparin content. This may represent a major extracellular function of mast cell granules and influence repair mechanisms within connective tissues.

Proteoglycan heparin is degraded within minutes of exposure to reactive radicals formed during the respiratory burst. The products have an approximate molecular weight of 12,000, which is similar to the size of heparins in commercial preparations. The cleavage product retains anticoagulent activity.

HL60 cells and their eosinophil- and neutrophil-like progeny all produce chondroitin 4-sulfates, but substantially differ in the rate at which they synthesize and degrade these molecules.

Human mast cells and elevated histamine levels can be found in the synovial fluid of patients having a wide variety of arthritides including rheumatoid arthritis, systemic lupus erythematosis, and osteoarthritis. These mast cells contain tryptase and appear to be connective tissue in type.

## Honors and Awards

Dr. Frank was invited to deliver the Enrique Ecker Lecture at Case Western University. He has been invited to deliver a lecture at the 600th Anniversary of Heidelberg University.

Dr. Warren Strober received the and Outstanding Service Medal from the United States Public Health Service.

Dr. Gallin received the Outstanding Service Award.

Dr. Straus was elected to the American Society for Clinical Investigation, the outstanding clinical investigative society in this country. He also received the Commendation Medal of the United States Public Health Service.

Dr. Metcalfe received the Commendation Medal of the United States Public Health Service.

Dr. Kaliner received the Outstanding Service Medal of the United States Public Heath Service.

Dr. Boltansky, in the Allergic Diseases Program, received a Travel Grant to the American Academy of Allergy and Immunology Meeting and a Travel Grant to the Aspen Allergy Conference.

Because of the continued development of Dr. Metcalfe's group during the year it was decided to elevate this group to a full section within the Allergic Diseases Program and this decision has now been implemented.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00043-20

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Dr. John E. Bennett, Head, Clinical Mycology Section, LCI, NIAID
Others: Dr. Ronald G. Washburn, Medical Staff Fellow, LCI, NIAID
Dr. Virginia Kan, Medical Staff Fellow, LCI, NIAID

COOPERATING UNITS (if any) Michael Mitchell, M.D., Micro. Lab., CC, NIH. Dennis George, DMB, DCRT, NIH. Martin Kroll, M.D., Clin. Chem., CC, NIH. David Klym, Beth. Naval Hosp. Michelle Evans, Univ. of NC, Chapel Hill. J.P. Pandey, Ph.D., Med. Univ. S.C., ORS, NIH, David Henderson, M.D., Hospital Epidemiologist, CC, NIH

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, N.I.H. Bethesda, MD

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.4 | 2.4 | 2.0 |

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

_Aspergillus fumigatus_ galactomannan was found by ELISA or RIA in urine from 7 of 8 patients with invasive aspergillosis caused by that species but not in urine from controls or from 2 patients infected with _A. flavus_. The assay appears useful in diagnosis of this potentially fatal infection.

_Aspergillus fumigatus_ was found to produce an extracellular substance which inhibits the ability of normal human serum to opsonize fungal cells for ingestion by human monocytes. The substance impairs the alternative pathway of the complement cascade, causing reduced deposition of C3b on the fungal surface.

A rapid enzymatic assay for the antifungal drug, flucytosine, was described. Creatinine iminohydrolase (EC 3.5.4.21) was used to quantitatively remove the amino group from flucytosine. Ammonia was then measured colorimetrically.

Otherwise normal patients who are cured of cryptococcosis are specifically tolerant to immunization with cryptococcal polysaccharide. Recent work has shown that immunization of volunteers but not cured patients leads to circulating B cells with surface anticryptococcal antibody, indicating that tolerance in these patients occurs at an earlier stage of the humoral response.

Immunoglobulin allotypes Gm 1,2,3,17;23,5,6,13,12, Km1 and Km3 did not correlate with immune response of normal volunteers to cryptococcal or pneumococcal polysaccharide.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00045-17 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| Others: | Andrea J. Tenner, Ph.D. | Senior Staff Fellow | LCI/NIAID |
| | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| | Thelma Gaither | Research Biologist | LCI/NIAID |
| | Lois Renfer | Chemist | LCI/NIAID |
| | Kathleen Melez, M.D. | Guest Scientist | LCI/NIAID |

COOPERATING UNITS *(if any)*
Neil Young, M.D. IR/CHB/NHI
Jeffrey Moore IR/CHB/NHI

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | .5 | .5 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


C1 inhibitor, a glycoprotein found in normal human serum, is a modulator of the activation of C1, the first component of the classical complement pathway, as well as an inhibitor of the activated enzymes C1r and C1s. The lack of this protein in serum either as an acquired condition or as a genetic deficiency usually results in chronic or acute angioedema. The goal of this research project is to investigate the physiologic fate of the complexes formed when C1 is activated either in the presence or absence of C1 inhibitor. Our initial efforts have focused on developing sensitive and if possible simpler, more precise hemolytic assays for both C1 inhibitor and proenzyme C1. By taking into account the binding affinities of C1 inhibitor for activated C1r and C1s, a more sensitive assay for C1 inhibitor was devised. Similarly , by modifying the conventional C1 hemolytic titer, a simple yet sensitive and quantitative assay was developed to differentiate unactivated (proenzyme) C1 from activated C1. In exploring the kinetics of the C1-C1 inhibitor interaction we observed that the rate of inactivation of activated $C1r_2s_2$ is dependent on the concentration of C1 inhibitor. In addition, our data demonstrate that cell bound C1 is less susceptible to inhibition by C1 inhibitor than is fluid phase activated C1. These findings suggest that those parameters of a substance that limit access of C1 inhibitor to the C1r and C1s enzymes may contribute to the definition of the substance as a C1 activator and may help to explain the seemingly unpredictable clinical manifestation of C1 inhibitor deficiencies.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00047-16 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. A. Ottesen, Head, Clinical Parasitology Section LCI/LPD, NIAID

Others: See Next Page

COOPERATING UNITS (if any)

See Next Page

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 3.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The goal of these studies is to increase understanding of the pathogenesis, and to improve diagnostic and therapeutic measures for parasitic diseases.

A method of purifying bovine cryptosporidial oocysts for use as an antigen in an ELISA test was developed. IgM and IgG antibody responses were demonstrated in immunocompetent as well as in AIDS patients, with indications the Cryptosporidium is a common human infection. The criteria and specificity for an immediate hypersensitivity skin test for strongyloidiasis have been established. For immunodiagnosis of filarial infections efforts to improve the sensitivity assays for circulating filarial antigens are continuing.

Therapeutic trials underway include a double-blind prophylactic trial of DEC against acquisition of Loa loa infection in Peace Corps volunteers in Africa, treatment of cerebral cysticercosis with praziquantel, treatment of strongyloidosis with Ivermectin, and use of ivermectin to treat lymphatic filariasis in India. Recombinant gamma interferon was found to show limited effects in treating anergic cutaneous leishmaniasis.

The relationship of the immune response to immunopathology in several types of helminthic and protozoan infection is being examined. The antigen-specific T-cell suppression in filariasis and leishmaniasis was demonstrated to be at the level of lymphokine (IL-2 and gamma-interferon) production. The relationship between IgE and $IgG_4$ generating and inhibiting allergic responses to parasites was aided by the use of anti-subclass monoclonal antibodies. Broncho-pulmonary lavage revealed persisting alveolitis in tropical pulmonary eosinophilia patients after therapy, suggesting that more intensive treatment may be necessary.

Giardia isolates from various sources are being characterized as to their DNA composition, surface antigens and biologic behavior. Several isolates will be used for human volunteer infections.




PHS 6040 (Rev. 1/84) GPO 914-918

OTHER PROFESSIONAL PERSONNEL
(name, title, laboratory, and institute affiliation)

| | | |
|---|---|---|
| T. E. Nash (Co-Principal Investigator) | Senior Investigator | LCI/LPD, NIAID |
| F. A. Neva (Co-Principal (Investigator) | Senior Investigator/ Chief | LCI/LPD, NIAID |
| R. Hussain | Sr. Staff Fellow | LPD, NIAID |
| A. Cheever | Asst. Chief | LPD, NIAID |
| D. Keister | Biologist | LPD, NIAID |
| L. Diamond | Head, Parasite Growth Section | LPD, NIAID |
| M. Lunde | Research Zoologist | LPD, NIAID |
| T. Nutman | Medical Staff Fellow | LPD, NIAID |
| R.G. Crystal | Chief | PB, NHLBI |
| W. London | Scientist | NIMH |
| D. Sacks | Staff Fellow | LPD, NIAID |
| P. Scott | Staff Fellow | LPD, NIAID |
| B. Unger | Medical Staff Fellow | LPD, NIAID |
| C. Maxwell | Medical Staff Fellow | LPD, NIAID |
| V. Kirchhoff | Medical Staff Fellow | LPD, NIAID |
| J. Sherwood | Medical Staff Fellow | LPD, NIAID |
| D. Ward | Medical Staff Fellow | LPD, NIAID |
| R. Davey | Medical Staff Fellow | LCI, NIAID |
| R. Lal | Visiting Fellow | LPD, NIAID |
| J. A. Dvorak | Research Microbiologist | LPD, NIAID |
| A. Aggarwal | Visiting Fellow | LPD, NIAID |
| C. Lane | Senior Investigator | LIR, NIAID |
| J. White | Medical Staff Fellow | LCI, NIAID |
| J. I. Gallin | Head, Cell Physiology Section | LCI, NIAID |

Cooperating Units:

University of Arizona, (E. Petersen); Peace Corps Medical Office, Washington, (K. Miller, R. Gibbs, N. Reinhart); Johns Hopkins University, (R. Hamilton); University of Michigan, (J. Bennett); University of Khartoum, Sudan (M. Homeida); Armauer Hansen Research Inst., Addis Ababa, Ethiopia, (G. Bjune); Meloy Labs, Springfield, Va., (G. Phillips); Harvard University, Boston, (F. Von Lichtenberg); Veterans Administration, Wichita, TX, (L. Pelletier); Onchocerciasis Chemotherapeutic Research Center, Tamale (K. Awadzi); Indian Council of Medical Research Tuberculosis Research Center, Madras, India (S.P. Tripathy, R. Prabhakar, P. R. Narayanan, V. Kumaraswami, R. Paranjape and V. Vijayan); Special Programme for Tropical Disease Research, WHO, Geneva; Centers for Disease Control (C. Reimer); Madras Medical College, Madras, India (K. Vijayasekaran); Tulane University (B. Cline, S. Katz and D. Little).

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00048-15 LCI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michael M. Frank, M.D. | Head, Clinical Immunology Section and Chief | LCI/NIAID |
| Others: | Jeffrey Moore | Biologist | CHB/NHLBI |
| | Neal S. Young, M.D. | Chief, Cell Biology Branch | CHB/NHLBI |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This year we have turned our attention to the disease Paroxysmal Nocturnal Hemoglobinuria. This disease is an acquired disorder in which red cells and other blood elements become abnormally sensitive to the lytic action of complement. It is believe that this represents a clonal disorder in which an individual clone of cells in the bone marrow becomes disordered, proliferates and takes over a portion of the marrow, forming cells with abnormal membranes. Others have shown in the past several years that these cells are missing a complement regulatory protein termed "decay accelerating factor" (DAF). Our preliminary experiments during the course of the last several years suggested to us that in fact bone marrow precursor cells which give rise to the cells which act as PNH cells do not have the PNH defect. This is a radical idea and represents an entirely new concept of the pathogenesis of PNH. To test the validity of this idea we have obtained bone marrow from patients with Paroxysmal Nocturnal Hemoglobinuria and use the fluorescent activated cell sorter with specific antibody to DAF to separate cells into precursors that have DAF and those that do not. The majority of the cells in the bone marrow in PNH patients are DAF negative--that is, abnormal cells. We find that all of the progeny that grow and therefore all of the cells that represent primitive stem cells are DAF positive. There are no stem cells in the DAF negative population. Moreover, direct test of the progeny of these DAF positive cells shows that many are DAF negative. The results indicate that the precursor cells in the bone marrow that give rise to PNH cells do not have the PNH defect. This represents a completely new concept of the disease.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00057-12

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic studies on pathogenic fungi

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

K.J. Kwon-Chung, Research Microbiologist, LCI/NIAID

William L. Whelan, Visiting Associate, LCI/NIAID

COOPERATING UNITS (if any)

P.T. Magee, Department of Microbiology and Public Health, University of Michigan, East Lancing, MI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Disease, NIH Bethesda, MD

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 2 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Topics of current studies include:(1) characterization of a new fungal pathogen isolated from a granulomatous lesion of olecranon bursa;(2) Pathogenesis and virulence factor of Candida albicans: (3) biochemical genetics of resistance to 5-fluorocytosine (5-FC) in C. albicans; (4) development of a parasexual genetic system for Torulopsis glabrata. An isolate of Anthopsis deltiodea was found to be the cause of an olecranon brusitis in a man. A. deltoidea has never been reported previously from clinical specimens. A. deltoidea produced dematiaceous hyphae in tissue in the center of the necrotic debris. The relationship between extracellular proteinase and virulence for mice in Candida albicans was studied using an isogenic set of proteinase - producing parent (C9), a proteinase - deficient mutant (C9M1) derived from the parent by nitrouse acid treatment and a spontaneous revertant (C9M1M) obtained by mouse passage of C9M1. The isolate C9 produced a high level of proteinase in vitro and caused 100% fatal infection within 21 days. The mutant produced no detectable enzyme in vitro and all mice survived until day 22. Only 30% of the mice infected with C9M1 died between day 23 and 30. The isolates recovered from the dead mice were found to be proteinase sufficient. The C9M1M produced proteinase in vitro at 44% of the level of the wild type and induced fatal infection in 90% of the mice within 30 days indicating that proteinase activity is one of the factors associated with the virulence of C. albicans. Resistance to 5-flucytosine in C. albicans results form a defect in UMP pyrophosphorylase activity (fcy1) or from a defect in cytosine deaminase activity (fcy2). We have demonstrated complementation in fcy1 FCY2 x FCY1 fcy2 crosses. A parasexual genetic system in Torulopsis glabrata was developed by spheroplast fusion between two haploid strains carrying different nutritional markers.



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**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00058-11 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: | S.E. Straus | Senior Investigator, LCI, NIAID |
| OTHER: | J. Rooney (until 6/85) | Medical Staff Associate, LCI, NIAID |
| | J. Felser | Medical Staff Associate, LCI, NIAID |
| | K. Croen | Medical Staff Associate, LCI, NIAID |
| | M. Sawyer | Medical Staff Associate, LCI, NIAID |
| | J. Ostrove | Senior Staff Fellow LCI, NIAID |
| | J. Wilkinson | Clinical Research Nurse, LCI, NIAID |

COOPERATING UNITS *(if any)*
G. Tosato, M. Blaese (MET/NCI)
S. Nusinoff-Lehrman (Burroughs Wellcome Company)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathogenesis, immunology, natural history and therapy of human herpesvirus infections are being investigated. Immunocompetent and immunodeficient patients, including those with AIDS, who possess a wide range of herpesvirus infections have been identified and studied. Our major emphases have been on frequently recurring genital herpes simplex virus infections and on chronic Epstein-Barr virus infection. During the past year we have completed a large longitudinal study of symptoms and virus shedding patterns in patients with frequently recurring genital herpes before, during and after suppressive acyclovir therapy. As part of that study we have identified and quantitated shedding of virus in the absence of symptoms. We have observed and studied an episode of transmission of genital herpes associated with an episode of asymptomatic shedding. In terms of chronic Epstein-Barr virus infection, we have continued long term observation of a series of patients with a chronic fatiguing illness associated with elevated Epstein-Barr virus antibody titers. We have initiated a placebo controlled trial of intravenous and high dose oral acyclovir therapy for patients with that disorder. During the coming year we plan to initiate another study of asymptomatic shedding in patients with genital herpes and to complete our current placebo controlled acyclovir trial for chronic EBV infections.



PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00154-10 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michael A. Kaliner, M.D. | Head, Allergic Diseases Section | LCI/NIAID |
| Others: | Thomas M. Keahey, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Jay E. Slater, M.D. | Medical Staff Fellow | LCI/NIAID |
| | William J. Meggs, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |
| | Susan L. Wescott | Biologist | LCI/NIAID |
| | Cynthia L. Murphy | Medical Technologist | LCI/NIAID |
| | Rosemary C. Pellicciotto | Medical Technologist | LCI/NIAID |

COOPERATING UNITS *(if any)*

Marc Friedman, Ph.D., Georgetown University (Contract# NO-1-A1-22665); Ruth Jacobs, M.D., Critical Care Medicine, Clinical Center; Kaspar Sertl, M.D., University of Vienna; and Martha V. White, M.D., Bureau of Biologics, FDA

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.9 | 1.9 | 3 |

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Our analysis of events in immediate hypersensitivity focuses on human and animal models of allergic responses, mechanisms of mediator action, and pharmacologic approaches to allergic diseases. The areas under investigation include asthma, allergic rhinitis, anaphylaxis, urticaria, and mastocytosis.

Employing monoclonal antibodies directed at cyclic GMP, the pattern of cells in guinea pig lung responding to histamine stimulation has been identified. Histamine causes all cells to increase their cytoplasmic cyclic GMP with an increased concentration near the nuclear membrane. In mouse lung, a population of dendritic cells has been identified which is found in the mucous membrane and is a potent antigen presenting cell. Ketotifen has been found to prevent histamine release from mast cells in patients with physical urticarias. Histamine levels in plasma are diagnostic of systemic mastocytosis if consistently elevated. The mechanism for progesterone-related anaphylaxis has been examined and remains unclear while a second progesterone-sensitive subject with anaphylaxis responded to LHRH analogue therapy. Microvascular permeability in skin is increased by histamine, serotonin, and bradykinin but does not appear to contribute to the edema seen in late phase responses. Plasma histamine from patients in gram negative sepsis and shock is reduced below normal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00155-10 LCI

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | John I. Gallin, M.D. | Chief, Bacterial Diseases | LCI/NIAID |
| Others: | Judith Falloon, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Daniel Rotrosen, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Bruce E. Seligmann, Ph.D. | Senior Staff Fellow | LCI/NIAID |
| | Jayasree Nath, Ph.D. | Expert | LCI/NIAID |
| | Yohichiroh Ohno, M.D., Ph.D. | Visiting Associate | LCI/NIAID |
| | Stephen C. Dreskin, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |
| | Cheryl Jo White, M.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS (if any)

| | |
|---|---|
| Michael M. Frank, M.D. | LCI/NIAID |
| John O'Shea, M.D. | LCI/NIAID |
| Thelma Gaither | LCI/NIAID |

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 10.42 | 7.42 | 3 |

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several aspects of phagocyte biology have been studied using neutrophils (PMN) and monocytes. Investigation of the subcellular location of fmet-leu-phe, C3bi as well as for cytochrome b suggests these constituents are packaged in a common compartment similar to specific granules. In related studies a series of monoclonal anitbodies against the inside of the neutrophil plasma membrane have been developed with the goal of identifying specific secretory granule attachment sites. Studies in exudate PMN have revealed greater than five-fold more fmet-leu-phe receptors than blood PMN, presumably related to receptors mobilized from the intracellular pool.

In studies extending our earlier work on neutrophil heterogeneity a monoclonal antibody, 31D8, that binds to a subpopulation of PMN was studied in chronic myelogenous leukemia (CML). The data indicate there are two groups of CML patients; those with 31D8 bright or dull PMN. It is not clear whether or not the CML heterogeneity reflects differences in clonal proliferation. Unexpectedly absence of the 31D8 antigen appeared predictive of progression of CML to the accelerated phase.

Studies of IgE turnover in the Hyperimmunoglobulin E-recurrent infection (Job's) syndrome have revealed decreased clearance of IgE in subjects with elevated IgE. This has implications for numerous diseases in which IgE is elevated.

In other studies gamma interferon was shown to be a chemoattractant for PMN and monocytes and this may relate to gamma interferon's ability to induce macrophage giant cell formation.



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Others (Continued):

| | | |
|---|---|---|
| Werner Zimmerli, M.D. | Guest Worker | LCI/NIAID |
| Joan Sechler | Technician | LCI/NIAID |
| Julia Metcalf | Technician | LCI/NIAID |
| Kerstin Cehrs | Technician | LCI/NIAID |

Cooperating Units (Continued):

| | |
|---|---|
| Eric Ottesen, M.D. | LPD/NIAID |
| Marc M. Friedman | Dept. Microbiol. Georgetown Med. Ctr. |
| Robert J. Jacobson | Dept. Medicine, Georgetown Med. Ctr. |
| Harry L. Malech, M.D. | Yale University, New Haven, CT |

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00189-06 LCI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical and Biochemical Studies of Human Enteral Adenovirus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Stephen E. Straus, Senior Investigator, LCI, NIAID

| OTHER: | | | |
|---|---|---|---|
| | J. Rooney (until 6/85) | Medical Staff Fellow | LCI, NIAID |
| | J. Ostrove | Senior Staff Fellow | LCI, NIAID |

COOPERATING UNITS *(if any)*

C. Brandt and W. Rodriguez (Children's Hospital, D.C.), H.S. Ginsberg (Columbia Univ.), R. Yolken (Johns Hopkins)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .75 | .5 | .25 |

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enteroadenoviruses (EAds) comprise a serogroup with two related types of adenoviruses which are associated with gastroenteritis in infants. We have continued our studies of the molecular biology and epidemiology of EAds. We have completed a study using highly specific cloned EAd DNA fragments for rapid diagnosis of infection. Early events in the replication of EAds in permissive and nonpermissive cell lines are being studied by binding of radiolabeled virus particles to cells and by characterization of early mRNA synthesis by Northern hybridization analysis.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00192-07 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Studies on Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principel Investigator.*) (*Name, title, laboratory, end institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Dean D. Metcalfe, M.D. | Senior Clinical Investigator | LCI/NIAID |
| Others: | William J. Meggs, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |
| | Daniel G. Malone, M.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS (*if any*)

Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (R. Wilder), The University of Washington (C.W. Henderson and S. Klebanoff), and Johns Hopkins University (T. Ishizaka)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.50 | 1.50 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Fibroblasts and endothelial cells both are capable of the phagocytosis and degradation of mast cell granules as demonstrated by microscopy and by the use of radiolabeled mast cell granules. Mast cell granules rapidly and selectively degrade extracellular fibronectin. While this degradation is due to chymase, mast cell granules are particularly efficient at cleaving fibronectin by virtue of their heparin content. This may represent a major extracellular function of mast cell granules and influence repair mechanisms within connective tissues.

Proteoglycan heparin is degraded within minutes of exposure to reactive radicals formed during the respiratory burst. The products have an approximate molecular weight of 12,000, which is similar to the size of heparins in commercial preparations. The cleavage product retains anticoagulent activity.

HL60 cells and their eosinophil- and neutrophil-like progeny all produce chondroitin 4-sulfates, but substantially differ in the rate at which they synthesize and degrade these molecules.

Human mast cells and elevated histamine levels can be found in the synovial fluid of patients having a wide variety of arthritides including rheumatoid arthritis, systemic lupus erythematosis, and osteoarthritis. These mast cells contain tryptase and appear to be connective tissue in type.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00249-04 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Dean D. Metcalfe, M.D. | Senior Clinical Investigator | LCI/NIAID |
| Others: | William J. Meggs, M.D., Ph.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS (*if any*)

Digestive Diseases Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (Jay Cherner)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Two forms of mastocytosis have been documented. Ninety percent of our patients have disease presenting initially as urticaria pigmentosa and which slowly progresses over decades. A second rapidly progressive form of mastocytosis presents with lymphodenopathy, peripheral eosinophilia, an elevated sedimentation rate, and an elevated alkaline phosphatase. One patient with this disease, which we have termed lymphadenopathic mastocytosis with eosinophilia, has had an initial response to a combination of cytoxan, vincristine, and prednisone. In a related observation, patients with malignancy and mastocytosis are more likely to have oligoclonal immunoglobulin bands on agarose gel electrophoresis, aiding in diagnosis.

Patients with systemic mastocytosis have elevated plasma histamine levels (approximately 2000 pg/ml). Patients with urticaria pigmentosa have slightly elevated plasma histamines, while patients with idiopathic anaphylaxis have normal histamines (approximately 270 pg/ml).

Systemic mastocytosis may be complicated by an increase in basal acid output, and by maladsorption. Such findings are highly variable, but tend to occur in patients with severe generalized disease.

The histamine content increases in cultures of human bone marrow in association with the appearance of poorly defined granulated cells which die out after approximately 6 weeks. Lectin-stimulated human peripheral mononuclear cells produce a factor which stimulates the growth of cultured, IL-3 dependent, mouse mast cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00250-04 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Dean D. Metcalfe, M.D. | Senior Clinical Investigator | LCI/NIAID |
| Others: | Daniel G. Malone, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Kim E. Barrett, Ph.D. | Fogarty Visiting Fellow | LCI/NIAID |
| | Ana Maria Saavedra-Delgado, M.D. | Medical Officer | LCI/NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.75 | 1.75 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


A comparison of lung mast cells with gastrointestinal mast cells from the same monkey reveals both similarities and differences. Both populations of cells degranulate to anti-human IgE, but not to compound 48/80. Degranulation induced by anti-IgE in both mast cell populations is inhibited by theophylline and quercetin. However, in comparison to gastrointestinal mast cells, lung mast cells stain more readily, have a higher histamine content, and release more mediators upon stimulation, confirming mast cell heterogeneity and the need to characterize mast cell populations in higher animals.

Cultured mouse mucosal mast cells degranulate upon exposure to N-acetyl cysteine, a mucolytic agent. Mucosal mast cell degranulation is inhibited by sulphasalazine. Aspartame has little or no direct effects on mast cells and basophils.

Patients with a history of immediate adverse reactions to foods and whose symptoms are reproduced on challenge, are atopic, have multiple positive skin tests to foods and inhalants, have a positive skin test to the food in question, and by history are those with the most severe reactions. Twenty-five patients with idiopathic anaphylaxis, and eight with systemic mastocytosis have been challenged with sulfites. No clinical reactions were observed, although plasma histamines were elevated following challenge. One severe reactions to sulfites was observed in an asthmatic.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 A1 00269-04 LCI

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Neutrophil Subpopulations

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Bruce E. Seligmann, Ph.D. | Senior Staff Fellow | LCI/NIAID |
| Others: | John I. Gallin, M.D. | Chief, Bacterial Diseases | LCI/NIAID |
| | Steve Dreskin, M.D. | Clinical Fellow | LCI/NIAID |
| | Thomas Chused, M.D. | Senior Staff | LMI/NIAID |
| | Richard Krochak, M.D. | Staff Fellow | LI/NIAID |
| | Reuben Siraganian, M.D. | Senior Staff | IRP/NIDR |

COOPERATING UNITS (*if any*)

| | |
|---|---|
| Millie Donlon, Ph.D. | Armed Forces Radiobiology Res. Branch |
| R. Jacobson, M.D. | Georgetown Univ. Hosp., Dept. of Med. |
| Harry Malech, M.D. | Yale University School of Medicine |
| Mark Fletcher, M.D. | University of California, Davis |

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1 | .5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


To investigate mechanisms of phagocytic cell activation we developed methods to measure intracellular calcium (fura2 & indol) and membrane potential independent of mitochondrial potential (oxonol dyes). A pool of intracellular calcium regulates neutrophil function, a calcium signal is sufficient to stimulate superoxide and secretion, and there is selective inhibition of calcium dependent activation by PMA, possibly through protein kinase c mediated phosphorylation. We studied protein kinase c using the activator di-C8 (diacylglycerol analog). di-C8 stimulates superoxide and secretion like PMA but low doses cause transient responses resembling chemoattractant effects.

We investigated the activation of mast cells and lymphocytes. Both calcium dependent and calcium independent activation mechanisms were found in mast cells. We found that a calcium dependent membrane potential depolarization was elicited by IgE binding. These changes reflect a large calcium efflux and its role in activation is now being defined using a series of cell lines with speific biochemical and functional defects. The studies with lymphocytes indicate that both T and B cells display a calcium dependent and activated potassium flux which is sensitive to manipulation in cold or minus calcium media. Studies with T cells and lines transvected with the T cell receptor indicate these cells are activated mitogentically and functional display a rise in calcium when the T cell receptor is crosslinked by antibody.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00270-04 LCI

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jayasree Nath, Ph.D. | Expert | LCI/NIAID |
| Others: | John I. Gallin, M.D. | Chief, Bacterial Diseases Sec. | LCI/NIAID |

COOPERATING UNITS *(if any)*

Dr. Cynthia Oliver, Laboratory of Biochemistry, NHLBI, NIH

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Bacterial Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 1.1 | |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The chemotactic peptide fmet-leu-phe and the $Ca^{2+}$-ionophore A23187, stimulate PMN tubulin tyrosinolation that is dependent on the presence of extracellular $Ca^{2+}$ and the activation of NADPH-oxidase mediated oxidative burst. Isolated PMN cytoplasts (CP) and karyogranuloplasts (KGP) fail to respond to either stimuli, indicating the requirement of an intact functional PMN for the modulation of PMN tubulin tyrosinolation. Unlike the specific stimulation of tubulin tyrosinolation in fmet-leu-phe or A23187-stimulated PMN, an intriguing phenomenon of tyrosine incorporation into multiple proteins was observed in PMA-activated PMN, that is dependent on the pathway for NADPH-oxidase activation and independent of protein synthesis. The reaction is inhibited by a variety of reducing agents and intracellular scavengers of oxygen radicals. PMA-activated PMN from patients with chronic granulomatous disease failed to exhibit this phenomenon, but activated PMN from a myeloperoxidase deficient patients incorporated the tyrosine. PMA-activation of PMN causes a two-fold increase in the generation of protein carbonyl derivatives, which is potentiated in the presence of labeled tyrosine. Reverse phase HPLC analysis of radiolabeled samples indicate the presence of radioactivity in multiple peaks with distribution throughout the protein (peptide) fractionation range. SDS-urea gel patterns also reveal similar results. The PMA-induced incorporation of tyrosine is highly exaggerated in KGP and also appears to be quite specific for tyrosine as other amino acids like phenylalanine, leucine, histidine or methionine, fail to incorporate. The biochemical mechanism and the functional role of this intriguing reaction remains to be elucidated.



PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00271-04 LCI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification and Characterization of Serum Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Andrea Tenner, Ph.D. | Staff Fellow | LCI/NIAID |
| | Lois Renfer | Research Biologist | LCI/NIAID |
| | Thomas Lawley, M.D. | Senior Investigator | DB/NCI |
| | Kim Yancey, M.D. | Med. Staff Fellow | DB/NCI |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 1.4 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Quantitative data from five preparations of C3 by our newly developed and shortened small scale procedure demonstrates the efficiency of recovery and preparation as well as purity of C3 isolated in this manner. C3 is prepared as described within three days as a fully active, homogeneous protein with recovery of over 70%. We have also shown that C4, C5 and C9 as well are obtainable by this protocol. Goat polyclonal, monospecific antisera to complement components C1s, C1q and FB as well as HSA has been or is being produced. Anti-C1s IgG coupled Sepharose will be used to stabilize purified preparations of C4. Anti-C1-In contaminants IgG (including anti-albumin and alpha-lipoprotein) was used coupled to Sepharose to remove small amounts of these contaminants from C1-In prepared by our recently developed isolation scheme. These contaminants originally identified by radioautographic analysis of $^{125}$I labelled C1-In have been effectively removed as evidenced by their absence in readsorbed and radioiodinated C1-In preparations. C2 isolated by a recently developed, rapid, 3 step procedure involving PEG precipitation, DEAE-Sephacel chromatography and functional affinity chromatography on C4b-Sepharose only needs quantitative analysis for completion. Extension of our new procedure for C5a purification from citrated human plasma has allowed for the isolation of C3a as well. The procedure incorporates C3a immunoadsorption of C5a depleted plasma by use of anti-C3a Sepharose. The acid-glycine eluted C3a is further purified by gel filtration on ACA-44 and concentrated by use of carboxymethyl cellulose. Final characterization of C3a prepared by this method including determination of its biological potency is planned.



PHS 6040 (Rev. 1/84) GPO 904-917

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00272-04 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Eric Brown, M.D. | Senior Investigator | LCI/NIAID |
| Others: | Richard Sveum, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Joseph Bekisz, M.S. | Microbiologist | LCI/NIAID |
| | Michael M. Frank, M.D. | Chief, Laboratory of Clinical Investigation | LCI/NIAID |

COOPERATING UNITS (if any)
Thomas Chused, LMI, NIAID

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.75 | 0.75 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Studies have been extended on neoantigens of immune complexed IgG by development of mouse monoclonal antibodies that react with the neoantigens. The monoclonals were developed in mice tolerized with monomeric or nonspecifically aggregated IgG. Several different monoclonal antibodies were characterized by their ability to recognize neoantigen or antigen bound IgG compared to monomeric plate bound IgG.

We have continued work on quantitative analysis of pneumococcal adherence and phagocytosis by human monocytes. The assay employs two fluorescent labels and dual laser flow cytometry. The pneumococci are labeled with Lucifer Yellow, which fluoresces independent of pH. Antibodies to pneumococcal capsular polysaccharides cleaved to $F(ab')_2$ fragments and biotinylated, then stained with Streptavidin-Texas Red were used to stain adherent but not ingested pneumococci. The assay has been used to study opsonization requirements for adherence and rate analysis of ingestion via the C3b receptor. Antibodies to Lucifer Yellow have been developed in rabbits which recognize the Lucifer labeled pneumococci without changing the Lucifer fluorescence.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00273-04 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Fibronectin in Opsonization and Phagocytosis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Eric Brown, M.D. | Senior Investigator | LCI/NIAID |
| Others: | John Bohnsack, M.D. | Medical Staff Fellow | LCI/NIAID |
| | George Martin, Ph.D. | Chief | LDBA/NIDR |
| | Hynda Kleinman, Ph.D. | Senior Investigator | LDBA/NIDR |
| | Gordon Laurie, Ph.D. | Visiting Fellow | LDBA/NIDR |

COOPERATING UNITS (if any)

Tsuneo Takahashi, American Red Cross

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been extended on the effects of laminin on phagocytosis. Using culture-derived human macrophages that are exposed to laminin we showed enhanced phagocytosis of EAC4b, EAC3bi and EA(IgG). The effect required interaction of laminin with the phagocytic cell and not opsonized particle. Direct comparison of the phagocytic ability of macrophages adherent to laminin and fibronectin coated glass slides showed that fibronectin had a greater effect on enhancing phagocytosis. This work shows the important stimulus of extracellular matrix proteins that activates macrophages normally present in the extracellular compartment and to the phagocytic cells that emigrate from the bloodstream to areas of inflammation.

New studies examined the interaction of Clq and laminin. Preliminary data suggests that laminin binds to the collagen-like tail of Clq via a short arm of laminin. The binding of laminin to Clq was stronger than the binding of Clq to fibronectin. A complex formed by laminin, Clq and aggregated IgG was dependent on the proportional amount of Clq bound to the aggregated IgG. Since laminin is found only in basement membranes, the interaction between laminin and Clq could be involved in the deposition and retention of immune complexes in these structures.



PHS 6040 (Rev. 1/84)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00275-04

PERIOD COVERED

October 1, 1985 - September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The Complement Receptor and C3 Mediated Opsonization

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Thelma Gaither | Research Biologist | LCI/NIAID |
| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Irma Vargas | Biologist | LCI/NIAID |
| | Shelley Wright | Chemist | LCI/NIAID |
| | Kevin Proctor | Laboratory Worker | LCI/NIAID |
| | John I. Gallin, M.D. | Section Head, BDS | LCI/NIAID |
| | Thomas Quinn, M.D. | Sr. Investigator | LCI/NIAID |

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2 | 1 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Polymorphonuclear neutrophils (PMN) from patients with chronic granulomatous disease (CGD), control patients with a variety of infections (inf. PMN) and PMN from one individual deficient in myeloperoxidase (MPO-def) were examined for phagocytosis in paired studies with normal healthy controls. Hyper-phagocytic activity was observed in PMN from 10 CGD patients and 12 infected patients as well as the patients with MPO deficiency. Normal PMN treated with sodium azide were also markedly more phagocytic than normal untreated PMN. Exogenously generated $H_2O_2$ caused a dramatic drop in phagocytosis by CGD and normal PMN while having no effect on MPO-Def. PMN. The $H_2O_2$ scavenger, catalase, also enhanced phagocytic activity in normal PMN. Thus MPO and $H_2O_2$ both play a role in regulating PMN phagocytosis. This effect appears to be mediated by regulation of the PMN Fc receptor since attachment of IgG coated particles, but not C3b coated particles, is also abrogated in normal PMN.

We showed previously that adhered cultured monocytes bind particles coated with the C3 degradation fragment, C3d, under certain conditions of activation and differentiation. We have also shown that, like the fragments C3b and iC3b, C3d enhances IgG mediated phagocytosis. Now we have clarified that the receptor involved in C3d binding is the iC3b receptor, CR3. Aparently monocyte CR3 expression changes in culture, allowing this receptor to interact with the C3d moiety of the C3 molecule.





PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00276-04 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Membrane Attack Complex of Human Complement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Keith Joiner, M.D. | Senior Investigator | LCI/NIAID |
| Others: | Martin Sanders, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Carl Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Stephen Puentes, M.D. | Med. Staff Fellow | LCI/NIAID |
| | Robert Scales | Med. Tech. | LCI/NIAID |

COOPERATING UNITS (if any)

Dr. Moon Shin, University of Maryland, Baltimore, MD
Dr. Lee Koski, University of Maryland, Baltimore, MD

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 1.0 | .2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Studies have continued on methods to quantitate formation of the membrane attack complex of complement. Antibody to neoantigenic determinants within polymerized C9 (anti: poly C9) has been extensively characterized and used to develop a sensitive ELISA for the fluid phase SC5b-9 complement complex. Cerebrospinal fluids from patients with a variety of central nervous system disorders were tested. SC5b-9 was detected in over 90% of spinal fluids from patients with Guillain Barre Syndrome and over 80% of spinal fluids from patients with multiple sclerosis but in 10% or less of spinal fluids from patients with non-inflammatory central nervous system disorders. Preliminary studies also document SC5b-9 in the CSF of several patients with systemic lupus erythematosis and central nervous system involvement. Anti-poly C9 antibody is currently being used in tissue immunofluorescence studies of diseased tissue from patients with rheumatoid arthritis, osteoarthritis and autoimmune dermatologic disease. Finally, anti-poly C9 was used to quantitate formation of C5b-9 complexes on complement-treated bacteria, in studies which demonstrated that multimeric C9 within C5b-9 was necessary for killing of a rough strain of E. coli.

Experiments have been initiated to study functional domains within the C9 molecule. Purified C9 has been biotinylated and then cleaved at a single site with alpha thrombin to produce a functionally active molecule with biotinylated hydrophobic and hydrophilic domains. Exposure of biotin within these domains to Avidin was tested for the monomeric C9 molecule and for C9 incorporated into C5b-9 in the fluid phase and on erythrocyte and bacterial membrane. We are now attempting to purify tryptic peptide fragments of C9 bearing biotin by reverse phase high performance liquid chromatography. These studies should provide a better understanding of the conformational changes within C9 which accompany formation of C5b-9.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00277-04 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Serum Resistance in Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
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| PI: | Keith A. Joiner, M.D. | Senior Investigator | LCI/NIAID |
| Others: | Carl H. Hammer, Ph.D. | Senior Investigator | LCI/NIAID |
| | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | Louis F. Fries, M.D. | Senior Investigator | LCI/NIAID |
| | Earl Bloch, Ph.D. | Visiting Scientist | LCI/NIAID |
| | Robert Scales | Med. Tech. | LCI/NIAID |

COOPERATING UNITS *(if any)*

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Peter Rice, M.D., Boston University School of Medicine, Boston, Mass.

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Laboratory of Clinical Investigation

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.3 | 1.1 | .2 |

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The complement requirements for killing of a rough, serum susceptible strain of E. coli (J5) were examined. Experiments showed that all five components of the terminal complex (C5b, C6, C7, C8 and C9) were required for killing. Furthermore, multimeric C9 within C5b-9 (C9:C5b-8 ratio of /3.3:1) was required for killing. This was shown in direct binding experiments with purified, radiolabelled C7 and C9, by measuring binding of an antibody specific for multimeric C9 to J5 bearing C5b-9, and by transmission electron microscopy of J5 outer membranes bearing C5b-9. Preliminary experiments measuring release of markers for J5 have shown that multimeric C9 within C5b-9 is required for release of the large periplasmic marker beta lactamase and the small cytoplasmic marker $^{86}$Rb. These experiments suggest that either C5b-9 bearing low C9 multiplicities does not have access to the cytoplasmic space or that the E. coli K+ transport systems compensate for C5b-9 channels until C9:C7 ratios of greater than 3.3:1 are achieved.

Experiments were continued on the mechanism of action of bactericidal antibody for E. coli 0111. Bactericidal antibody did not change the distribution of C3 on the capsule and outer membranes. However, nearly 1/5 of C3 deposited in the presence of IgG bound covalently to the antibody molecule. We therefore prepared covalent complexes of C3b-IgG. These complexes were 3-5 fold more efficient than IgG in presensitizing 0111 for direct complement killing, suggesting that formation of C3b-IgG complexes may be critical for the serum bactericidal reaction.

The mechanism of action of blocking IgG for Neisseria gonorrhoeae was tested. Results showed that blocking IgG enhanced rather than blocked complement consumption and desposition on GC. Furthermore, blocking IgG competed with bactericidal IgG for binding to GC. Finally, we showed that blocking IgG led to deposition of C3 at new sites on the outer membrane, sites which do not support formation of a bactericidal C5b-9 complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00278-04 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
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| Others: | Michael M. Frank, M.D. | Clinical Director | LCI/NIAID |
| | John Bennett, M.D. | Senior Investigator | LCI/NIAID |
| | Ronald Washburn, M.D. | Med. Staff Fellow | LCI/NIAID |

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Dr. D.L. Hoover, Dept. of Immunology, Walter Reed Army Med. Ctr.

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Laboratory of Clinical Investigation

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Clinical Immunology Section

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NIAID, NIH, Bethesda, Maryland

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|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treatment of $^3$H-arachidonic acid ($^3$H-C20:4)-labelled, antibody-sensitized mouse resident peritoneal macrophages with rabbit serum complement (C), or C6-deficient rabbit serum (C6D RS)+C6 caused hydrolytic release of incorporated $^3$H-C20:4 from phospholipids. High performance liquid chromatography revealed that the major radiolabelled products were C20:4, 6-keto-prostaglandin F1 alpha (6-k-PGF1 alpha) and prostaglandin $E_2$. The time-course of production of 6-k-PGF1 alpha, measured by radioimmunoassay (RIA), was biphasic. The dose response curve of production of 6-K-PGF1 alpha with respect to C rose to a peak at a dose producing 60% cell lysis. C6D RS produced only small quantities of this metabolite but reconstitution with purified C6 increased release substantially. Thus, C5b-9 may play a significant role in generation of C20:4 and its metabolites. L. major is an intracellular parasite of macrophages in mammalian hosts in which the amastigote form is destroyed with less than 1% normal human serum. Its killing is mediated by the alternate complement pathway in which the lethal process is initiated within 30 sec of exposure to serum. Using human sera genetically deficient in terminal complement components we investigated the requirement for C5 through C9 in the cytolytic process. We found that the degree of cytotoxicity was related to both serum concentration and to the point at which the deficiency occurred and indicate than an incomplete membrane attack complex may mediate cytotoxicity for this parasite. Human peripheral blood monocytes (PBM) ingest and kill Asperigillus fumigatus (AF) conidia and Cryptococcus neoformans (CN) by a C3b dependent opsonization in normal human serum (NHS). Cultures of AF produce a metabolite (IO) that inhibits opsonization and killing of CN by preventing deposition of C3b to their surface. Experiments using both $^{125}$I-anti C3b or $^{125}$I-C3 demonstrated decreases of up to 80% C3b binding using 10% AF IO. IO had little direct effect on C3 or C4 levels and only C3 and not C4 levels were depressed (70%) from supernatants obtained from mixtures of opsonized CN.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00279-04 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Kaliner, M.D. Head, Allergic Diseases Section LCI/NIAID

COOPERATING UNITS (if any)

James Shelhamer, M.D., Carolea Logun, and Jens Lundgren, Critical Care Medicine, Clinical Center

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NIAID, NIH, Bethesda, Maryland 20205

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| 0.1 | 0.1 | 0 |

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to products generated by pulmonary macrophages and peripheral mononuclear cells with increased mucous glycoprotein secretion. The macrophage and mononuclear derived secretagogues are collectively being called macrophage/mononuclear cell derived mucus secretgogues (MMS).

Activation of complement leads to anaphylatoxin generation. Current studies indicate that anaphylatoxins may be formed in pulmonary inflammatory processes. Therefore, the effects of human C3a upon mucus release were examined. C3a (and C5a) cause a dose-related stimulation of mucus secretion, maximal at 1-4 hours, apparently not requiring mast cell activation and not reproduced by C3a des arg. Thus, complement derived anaphylatoxins may also participate in mucus secretion.

Corticosteroids inhibit MGP release by lowering baseline secretion. Analysis of corticosteroid treated airways reveals a close correlation between lipomodulin generation and MGP production.

Pulmonary inflammation with neutrophils is often associated with mucus production. Lysates of human neutrophils as well as supernatants from activated neutrophils cause airways to release MGP; this activity is not due to elastase, and identity of the mucus secretagogue is under study.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00354-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Defects in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI/NIAID

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

Claudio Fiocchi, M.D., Cleveland Clinic Foundation, Cleveland, Ohio.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

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|---|---|---|
| 1.2 | 0.7 | 0.5 |

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this investigation is to define the T lymphocyte subpopulations and function of peripheral blood and intestinal lamina propria lymphocytes in patients with Crohn's disease, an idiopathic intestinal inflammatory disease which is thought to be immunologically mediated.

Lymphocytes were obtained from peripheral blood or isolated by an enzymatic digestion method from surgically resected intestinal specimens. Lymphocytes were then examined by dual immunofluorescence using a dual laser flow cytometer, using combinations of monoclonal antibodies which have been shown to correlate with different lymphocyte functions.

In peripheral blood, patients with Crohn's disease have a similar proportion of lymphocytes in the suppressor-inducer subset, in the suppressor-effector subset, and in the cytolytic T cell subset compared to controls. Interestingly, patients with Crohn's disease and controls had a significantly decreased proportion of lymphocytes having the phenotype of suppressor-inducer cells and the phenotype of suppressor effector cells. These results indicate that the intestinal lamina propria contains distinctly different subpopulations of T cells compared to peripheral blood which are presumably involved in mucosal host defense mechanisms. Further definition of the function of intestinal lymphocytes will provide greater under standing of the mechanisms involved in intestinal inflammation in inflammatory bowel disease.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00355-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory defects in primary biliary cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI/NIAID

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

E. Anthony Jones, M.D., Chief, Section on Liver Diseases, DDB/NIADDK

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National Institute of Allergy and Infectious Diseases, NIH, Bethesda MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.4 | 0 |

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XX (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


We have carried out a series of studies in patients with primary biliary cirrhosis (PBC), an idiopathic disease characterized by inflammation and necrosis of intrahepatic bile ducts, often associated with systemic autoimmune features.

To study the possibility that autoimmune features of PBC may be due to B cell abnormalities, we studied B cells for evidence of activation. PBC patients had an increased proportion of a normally very small population of circulating immunoglobulin secreting cells in peripheral blood. However, the majority of B cells were found to lack increased expression of transferrin receptor, which we have shown to be associated with B cell activation. These findings indcate that only a small population of activated B cells may contribute to the autoimmune process.

In other studies we found that patients with PBC have deficient natural killer cell activity in peripheral blood, which was shown to be due to deficient lytic activity of NK cells. The deficiency of NK activity in PBC may reflect a more generalized abnormality related to other previously described lymphocyte abnormalities in this disease.

Finally, we studied a family in which multiple patients had PBC, liver disease, and autoimmunity, and found one family member with selective IgA deficiency. This finding indicates that the IgA immune system, of which the hepatobiliary system is a part, is not necessary for the pathogenesis of PBC.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00356-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Regulation of IgA Immuhoglobulin Synthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Warren Strober, M.D. | Chief, Mucosal Immunity Section | LCI, NIAID |
| OTHER: | David Jacobs, M.D. | Staff Associate, MIS | LCI, NIAID |
| | Michael Sneller, M.D. | Staff Associate, MIS | LCI, NIAID |

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD. 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.3 | 2.3 | .5 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The overall objective of this project is to define the cellular and molecular events which underlie the differentiation of B cells into IgA-bearing and secreting B cells. In previous studies, it was shown that T cell clones derived from Peyer's patches had the capacity to induce IgM-bearing B cells (in the presence of LPS) to differentiate into IgA-bearing B cells. The present series of studies were undertaken to define the molecular events which accompany T cell-induced B cell switches, particularly IgM IgA switches.

Initially, we developed a series of T cell hybridomas from Peyer's patches and spleen by fusing Lyt-1$^+$ T cells with BW5147 mouse tumor cells. We then determined the capacity of selected hybridomas to cause differentiation of various clonal B cell lines. We found that the pre-B cell line, 70 Z/3, exhibited only small amounts of surface IgM and no surface IgG or IgA when carried in culture; however, following stimulation with LPS,surface IgM increased dramatically. In contrast, treatment of 70 Z/3 B cells with LPS in the presence of any of several (but not all) PP-derived T cell hybridomas as well as several spleen-derived T cell hybridomas, substantial amounts of IgG (as well as IgM) appeared on the cell surface. In addition, in the case of one PP-derived T cell hybridoma, IgA also appeared on the cell surface. Similar results (i.e., appearance of surface IgG) was obtained when 70 Z/3 B cells were co-cultured with selected T cell hybridomas and IL-1 alone or anti- sepharose beads plus IL-1. Examination of cells for their content of Ig specific m-RNA disclosed that LPS-stimulated 70Z/3 B cells,but not unstimulated 70Z/3 B cells contain $\gamma$ and $\alpha$ -mRNA but do not show rearrangement of $\gamma$ and $\alpha$ heavy chain DNA segments. These results show that T cells derived from the Peyer's patch can induce changes in Ig-class expression in the pre-B cell line 70 Z/3; these findings are consistent with either true Ig switches or, alternatively, increased transcription of pre-existent IgG m-RNA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00357-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
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| P.I. | Warren Strober, M.D. | Chief, Mucosal Immunity Section | LCI,NIAID |
| OTHER: | Hiroyuki Kotani, M.D. | Visiting Scientist, MIS | LCI,NIAID |

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Hiroaki Mitsuya, M.D., Visiting Scientist, Clinical Oncology Program, NCI, NIH

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| 1.3 | 1.3 | 0 |

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is directed toward the study of the autologous mixed lymphocyte reaction (AMLR), the proliferative response of T cells brought about by their exposure to autologous B cells and macrophages. In the present series of studies we report on the establishment of autoreactive T cell clones and the nature of the immunoregulatory capacity of such clones. In initial studies we found that autoreactive T cells develop in cultures repetitively stimulated by antigen (tetanus toxoid) presented by autologous non-T cells (in the presence of IL-2). Such cells appear along with antigen-reactive T cells, but can be isolated from the latter and then expanded using limiting dilution techniques. One of the autoreactive clones, termed MTC-4, has the phenotype of "helper" cell (Leu 3+, Leu 2$^-$) and undergoes proliferation when co-cultured with autologous, but not allogeneic non-T cells. Of interest, the immunoregulatory potential of MTC-4 cells varied according to how the cells were activated. When MTC-4 cells were cultured with autologous non-T cells in the absence of antigen or mitogen, polyclonal immunoglobulin production was observed. This helper activity was MHC-restricted in that it was elicited only by autologous non-T cells or MHC matched allogenic non-T cells; however, once activated by autologous non-T cells, MTC-4 cells could also help allogeneic non-T cells. In contrast, when MTC-4 cells were cultured with autologous non-T cells in the presence of pokeweed mitogen (PWM),immunoglobulin production was suppressed. This suppression was not due to a direct effect of PWM on MTC-4 cells, since preincubation of the latter with PWM prior to culture with non-T cells did not result in suppression. On the basis of these data, we conclude that autoreactive T cells have dual regulatory capability which is differentially elicited by the mode of activation: 1) when stimulated by MHC antigens present on unactivated B cells, they provide helper activity; and 2) when stimulated by MHC antigens present on activated B cells,they provide suppressor activity. Autoreactive cells with these properties are uniquely adapted to maintain immunologic homeostasis.



PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00358-03 LCI |
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PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunopathogenesis of Chlamydia trachomatis Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Thomas Quinn, M.D. | Senior Investigator | LCI, NIAID |
| Others: | Steven James, M.D. | Senior Investigator | LCI, NIAID |
| | Warren Strober, M.D. | Chief, Mucosal Immunity | LCI, NIAID |

COOPERATING UNITS (if any)
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University of California: Julius Schacter

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

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☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

**Chlamydia trachomatis** is the most common sexually transmitted bacterial pathogen in the United States. Studies have been carried out to further define the clinical spectrum of disease associated with chlamydia, to develop rapid diagnostic assays for screening and to examine the immunopathogenesis of chlamydial infection.

**Spectrum of chlamydial infection.** We instituted clinical studies to determine the prevalence of chlamydia infection in pregnant women and to determine its association with several clinical syndromes. In a survey of 1450 pregnant women, C. trachomatis was identified by cervical culture in 14.6%. There was a significant correlation with chlamydia infection and younger age (less than 20 years of age), and with cervicitis ($p < 0.0001$). In addition, chlamydia was found to be significantly associated with prematurity of the infants, and postpartum or postabortal endometritis ($p < 0.05$).

**Development of rapid diagnostic assays.** In a comparison of 2250 patient specimens, we demonstrated that detection of C. trachomatis by means of a direct immunofluorescent monoclonal antibody test is both sensitive (90%) and specific (98%) when compared to culture. In addition, it was more reliable than routine cytologic pap smear diagnosis of C. trachomatis. Detection of chlamydia by an in situ DNA hybridization method developed within our laboratory was also found to be both sensitive (91%) and specific (80%) when compared to culture, and may also be used for the screening of high risk individuals.

**Immunopathogenesis.** Using a primate model of rectal LGV infection, acute chlamydial infection was established in 10 cynomolgus monkeys and followed prospectively for 12 weeks. LGV rectal infection was correlated with reversal of systemic T cell lymphocyte populations and in alterations of mucosal natural killer cell populations. These changes in the immune response coincided with periods of highest infection burden, and the development of reactive lymphoid follicular hyperplasia. The above studies demonstrate the wide clinical spectrum and associated morbidity with C. trachomatis infection and provide methods for rapid screening of chlamydia and for studying its immunopathogenicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00359-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of ELISA Assays For Intestinal Protozoans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Thomas Quinn, M.D. | Senior Investigator | LCI, NIAID |
| Others: | Theodore Nash, M.D. | Senior Investigator | LCI, NIAID |

COOPERATING UNITS *(if any)*

Johns Hopkins University School of Medicine: Robert Yolken, Beth Ungar

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0 | 0 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00360-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reticuloendothelial Function In Patients With AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Thomas Quinn, M.D. | Senior Investigator | LCI, NIAID |
| Others: | Michael Frank, M.D. | Lab Chief | LCI, NIAID |
| | John Bohnsack, M.D. | Clinical Staff Fellow | LCI, NIAID |

COOPERATING UNITS *(if any)*

National Institute on Aging: Bradley Bender, M.D.
National Cancer Institue: T. Lawley, M.D.

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.4 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


**The acquired immune deficiency syndrome (AIDS)** is characterized by multiple opportunistic infections which are normally killed by phagocytic cells of the reticuloendothelial system (RES). We have prospectively analysed in vivo RES function by measuring Fc receptor clearance and C3b-receptor clearance in AIDS patients and patients at risk for AIDS.

**In vivo RES clearance defects.** A total of 63 patients have been studied, including 22 AIDS patients, 9 patients with AIDS-related complex, 13 healthy homosexual men, 15 healthy heterosexuals and 4 heterosexuals with mycobacterial disease but without AIDS. Eleven of fifteen AIDS patients and 2 of 9 with AIDS-related complex had prolonged Fc specific clearance rates compared to controls. In contrast, patients with mycobacterial infection but without AIDS had significantly more rapid clearance rates reflective of activated macrophage function. Similarly, C3b clearance rates were markedly abnormal in 7 AIDS patients compared to controls. Patients with AIDS had a relatively large initial clearance of C3b labeled cells, but unlike controls, they had a relatively large release of cells back in circulation. These defects of both Fc and C3b clearance rates suggest a marked phagocytic defect of the RES.

**In vitro phagocytic defects.** In order to measure the degree of phagocytic defect, we investigated antibody directed cell mediated cytoxicity (ADCC) of peripheral blood mononuclear cells of patients with AIDS against chicken red blood cells (CRBC). Compared to healthy controls, a marked decrease in ADCC-CRBC activity was observed in mononuclear cells from AIDS patients. No suppression in ADCC activity was seen when mononuclear cells from healthy subjects were assayed using media containing 10% sera from AIDS patients.

The **significance** of this project is in the identification of a marked phagocytic defect both in vivo and in vitro in patients with AIDS. Further studies will examine the severity and characterization of the phagocytic defect.




PHS 6040 (Rev. 1/84)

GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00361-03 LCI |
|---|---|

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic, Immunologic and Virologic Features of AIDS in Africa

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Thomas Quinn, M.D. | Senior Investigator | LCI, NIAID |
| Others: | Henry Francis, M.D. | Expert Scientist | LIR, NIAID |
| | Thomas Folks, M.D. | Senior Investigator | LIR, NIAID |
| | Anthony Fauci, M.D. | Lab Chief | LIR, NIAID |
| | Malcolm Martin, M.D. | Lab Chief | LMV, NIAID |

COOPERATING UNITS *(if any)*

Centers for Disease Control: J. McCormick, J. Curran, J. Mann
The Tropical Medicine Institute of Antwerp, Belgium: Peter Piot

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Over 12,000 cases of the Acquired Immune Deficiency Syndrome (AIDS) have been diagnosed in the US resulting in over 5,000 deaths. Prospective studies have been undertaken in Central Africa to study the unique epidemiologic, virologic and immunologic features of the disease which has occurred in several thousand Africans.

**Epidemiologic features.** Over 400 cases of AIDS has been identified in Kinshasa over the past 8 months (incidence rate: $380/10^6$). Unlike the US, the male to female ratio is 1:1, and the disease is predominately transmitted heterosexually. Household transmission studies demonstrate that 72% of spouses of AIDS patients were infected with HTLV-III virus compared to 4% of control households. Excluding spouses, 11% of household members of cases were seropositive compared to 3% of control households ($p < 0.05$), suggesting other possible means of transmission. Studies of blood bank donors, health care workers, hospitalized pediatric patients and neonates suggests both vertical and needle transmission.

**Virologic features.** The ELISA for detection of antibody to HTLV-III has proven to be both sensitive (99%) and specific (99%) in studies of over 400 African AIDS patients and 5,000 healthy African controls. HTLV-III has been isolated from 27 of 35 (77%) patients with AIDS and from nearly 70% of seropositive non-AIDS patients. Genomic studies of Zairian isolates demonstrate marked heterogeneity compared to North American and European isolates. Further studies will examine differences in human and animal viral isolates in Kenya and Zaire in order to study viral heterogeneity, and its relationship to clinical and immunologic features.

**Immunologic features.** Seropositivity to HTLV-III correlated with marked depression of T4 + and T8 + lymphocytes and anergy. Patients with tuberculosis and malaria have increased activated T cell and a high prevalence of HTLV-III antibody (40% and 20%, respectively). Seropositivity in these diseases correlated with elevated T8 + cells followed by depressed T4 + cells. Further studies will examine whether endemic tropical diseases may increase susceptibility to HTLV-III infection and/or accelerate the development of opportunistic infections among seropositive individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00379-03 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Studies of DNA Viruses and Other Possible Agents in AIDS Patients

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: S. E. Straus, Senior Investigator, LCI, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0 | 0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Project terminated.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00396-02 LCI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Complement Receptors: Regulation of Expression and Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.J. O'Shea | Medical Staff Fellow | LCI/NIAID |
| Others: | E.J. Brown | Senior Investigator | LCI/NIAID |
| | R.J. Sveum | Medical Staff Fellow | LCI/NIAID |
| | J. Bohnsack | Medical Staff Fellow | LCI/NIAID |
| | T. Gaither | Biologist | LCI/NIAID |
| | M.M. Frank | Clinical Director, NIAID | LCI/NIAID |
| | T. Lawley | Senior Investigator | NCI |

COOPERATING UNITS *(if any)*

| | |
|---|---|
| M. Berger | Case Western Reserve University, Cleveland, OH |
| T. Takahashi | American Red Cross |
| K. Yancey | ·USUHS |

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL. | OTHER: |
|---|---|---|
| 1.00 | 1.00 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Human phagocytes have at least two classes of receptors that mediate phagocytosis of opsonized particles. While Fc receptors constitutively mediated phagocytosis, the function and membrane expression of complement receptors are under regulatory control. Considerable progress has been made in the last year in understanding the mechanisms involved in control of plasma membrane expression and function of these receptors.

We first showed that a variety of cell activators induce upregulation of complement receptors. Intracellular latent pools of receptors exist for complement receptors and the intracellular location of one class of complement receptors was elucidate. Receptor upregulation was also noted to be dependent upon calcium mobilization. Regulation of receptor expression per se did not enable cells to mediate phagocytosis.

We also found two proteins that modify the phagocytic function of complement receptors. We studied the effect of one of these proteins, fibronectin as well as phorbol esters the behavior of complement receptors in neutrophils. Phorbol esters but not fibronectin induce ligand-independent internalization of CR1 by a cytoskeletal and temperature dependent mechanism. These agents also perturbate the association of CR1 with cytoskeleton. Synthetic diocylglycerol also induce receptor internalization. Both phorbol esters and synthetic diacylglycerols augment phagocytosis even through plasma membrane expression of CR1 is decreased. We also studied the role of calcium in these processes.

We propose that the physiologic activation of CR1 may occur via polyphosphoinositide metabolism through the activation of protein kinase C and calcium mobilization. Understanding the regulation of function of complement receptors is of importance both from the point of view of cell biology as well as disease pathogensis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00397-02 LCI

PERIOD COVERED
October 1, 1984 - September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interactions of C3b with Immunoglobulin G - Regulation of C3b Function by Antibody

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Louis F. Fries, III, M.D. | Senior Staff Fellow | LCI/NIAID |
| Others: | Keith A. Joiner, M.D. | Senior Investigator | LCI/NIAID |
| | Alejandro Malbran, M.D. | Fogarty Fellow | LCI/NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that C3b resides in a protected site when it is covalently bound to IgG (C3b-IgG). Such C3b displays a reduced affinity for factor H, with consequent enhnaced survival in the presence of factors H and I. Since erythrocyte CR1 may be a major co-factor for factor I-mediated inactivation of immune complex-borne C3b in blood, we have examined the effect of covalently bound IgG on the C3b-CR1 interaction. Binding of monomeric C3b and C3b-IgG to human erythrocyte CR1 demonstrates identical ionic strength dependence for both species. Identical numbers of binding sites with indistinguishable affinities are detected by both ligands. Cleavage of the ' chain of C3b and the '-heavy chain of C3b-IgG proceeds at the same rate when CR1 serves as co-factor for factor I. CR1 supports a second cleavage of fluid phase iC3b $_1$ chain that generates C3c and a 33,000 m.w. fragment, which bears antigenic markers characteristic of C3g. Inactivation of C3b and C3b-IgG by CR1 and factor I can occur at physiologic ionic strength, but proceeds slowly relative to rates attainable with sub-physiologic inputs of factor H. Thus, inactivation of C3b-IgG hetero-dimers or small immune complexes bearing limited numbers of C3b residues may remain largely factor H-dependent in vivo, with resultant enhanced C3b survival.

Since coating of bacteria with specific IgG antibodies enhances complement-mediated bacterial killing and simultaneously provides the opportunity for the formation of C3b-IgG, we additionally studied the role of C3b-IgG in complement-mediated bacterial lysis. Bacteria were coated with C3b alone, C3b followed by specific IgG, or preformed covalent hetero-dimers of C3b and specific IgG. The latter species demonstrated a 4 to 10 fold enhancement of its capacity to support alternative-pathway bacterial killing. This effect was not dependent on aggregation or total levels of C3b uptake--and may represent a new and important role for IgG in enhancing serum-mediated killing of gram-negative bacillary pathogens.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00398-02 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Interaction of Complement and Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Keith Joiner, M.D. | Senior Investigator | LCI/NIAID |
| Others: | Alan Sher | Chief, Immunology & Cell Biology Section | LPD/NIAID |
| | L.V. Kirchhoff | Medical Staff Fellow | LPD/NIAID |
| | Stephen Puentes | Medical Staff Fellow | LCI/NIAID |
| | Dennis Dwyer | Senior Investigator | LPD/NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.8 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Studies on the C3 acceptor molecule were extended from earlier work with Epimastigotes (Epi) of *T. cruzi* to culture derived metacyclic trypomastigotes (CMT) of *T. cruzi*. C3 binding studies showed that nearly five fold less C3 bound to CMT than to Epi during incubation in serum. GP72 on CMT, which is the predominant surface-iodinatable constituent on this life cycle stage, is an inefficient acceptor for C3 deposition. This is in contrast to Epi for which GP72 is the preferential C3 acceptor during incubation in serum.

We next examined the molecular basis for the difference in alternative pathway activation on Epi and CMT. First, the form of C3 fragments on Epi and CMT during serum incubation was examined. Approximately 3/4 of C3 on Epi following a 60 minute incubation was present as C3b, and the remainder was present as iC3b. In contrast, 85%-90% of C3 on CMT was present as iC3b. Purified factors B,D,P and C3 were used to deposit C3b on Epi and CMT and saturation binding studies of $^{125}$I B and $^{125}$I H were done on Epi and CMT bearing C3b. These studies showed that H binding curves were biphasic and nearly identical on both Epi and CMT, with high and low affinity populations. In contrast, although B binding was monophasic and of high affinity on Epi, B binding was biphasic and of markedly decreased affinity and extent on CMT. We have concluded from these studies that control of alternative pathway activation in Epi and CMT of *T. cruzi* is exerted at the level of B binding, and presumably reflects either the known difference in the C3 acceptor on Epi and CMT or the presence of regulatory molecules that differ between these two life cycle stages.

Preliminary experiments are underway to investigate the interaction of C3 with promastigotes (P) of *L. donovani*. Results show that C3 deposits on P via antibody-independent alternative pathway activation, that C3 binds covalently to a high molecular weight parasite constituent, and the majority of C3 is present as C3b.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00428-01 LCI |
|---|---|

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

IgE Immunotoxins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michael A. Kaliner, M.D. | Head, Allergic Diseases Section | LCI/NIAID |
| Others: | Howard Boltansky, M.D. | Senior Staff Fellow | LCI/NIAID |
| | Jay E. Slater, M.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS *(if any)*

Chaviva Isersky, Ph.D., National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases; and Richard Youle, Ph.D., National Institute of Neurological and Communicative Disorders and Stroke

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.9 | 1.9 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mast cells are the cellular nidus of allergic diseases and the cell responsible for disease in urticaria pigmentosa and systemic mastocytosis. This project is designed to ablate mast cells by attaching cytotoxic agents to IgE or antigen and selectively introducing the toxic product into mast cells. IgE linked to ricin kills RBL cells spontaneously and after crosslinking the IgE with anti-IgE. IgE linked to ricin's A chain kills only in the presence of monensin, a carboxylic ionophore. Therefore, IgE-linked immunotoxins are a new and possibly useful way to ablate mast cells.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00429-01 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Studies on Nasal Responses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michael A. Kaliner, M.D. | Head, Allergic Diseases Section | LCI/NIAID |
| Others: | Howard M. Druce, M.D. | Visiting Associate | LCI/NIAID |
| | Gordon D. Raphael, M.D. | Medical Staff Fellow | LCI/NIAID |

COOPERATING UNITS (*if any*)

Robert F. Bonner, M.D., Biomedical Engineering and Instrumentation Branch, Division of Research Services

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Allergic rhinitis is the most common chronic condition suffered by Americans, with about 7% of the population involved. Until recently, few studies of nasal physiology were being performed in this country. We are examining the effect of mediators and anaphylaxis on nasal blood flow and protein secretion by comparing atopic humans to normal controls. Nasal blood flow is not affected by methacholine but is reduced by alpha adrenergic agonists. Protein secretion is increased by methacholine and histamine in all subjects, but atopics are far more reactive than control populations. We are currently studying antigen challenge in these same populations.



PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00430-01 LCI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Varicella-Zoster Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: | S.E. Straus | Senior Investigator, LCI, NIAID |
| OTHER: | J. Felser | Medical Staff Associate, LCI, NIAID |
| | K. Croen | Medical Staff Associate, LCI, NIAID |
| | M. Sawyer | Meeical Staff Associate, LCI, NIAID |
| | J. Ostrove | Senior Staff Fellow, LCI, NIAID |

COOPERATING UNITS *(if any)*

J. Hay (USUHS), W. Ruyechan (USUHS)

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Medical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 3.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our major laboratory focus on the study of the molecular biology and latency of varicella-zoster virus (VZV) has continued. We are performing finer endonuclease mapping of varicella zoster virus DNA strains as well as completing a preliminary map of 58 viral encoded transcripts. We are using our extensive library of VZV DNA recombinants to map various VZV encoded gene products. Using marker rescue techniques we are attempting to map the genetic loci associated with VZV resistance to antiviral drugs. By hybrid selection and in vitro translation of viral RNAs and immunoprecipitation with polyclonal and monoclonal antibodies we are identifying and mapping major viral proteins. By tranasformation of thymidine kinase deficient mouse L cells we have identified and mapped the gene responsible for the VZV pyrimidine kinase enzyme. During the coming year we plan to initiate in situ hybridization studies of human tissues for latent VZV DNA and RNA sequences.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00432-01 LCI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of mucosal immune responses in non-human primates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen P. James, M.D., Investigator, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205.

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.9 | 0.5 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The purpose of this investigation is to characterize the phenotypes and function of intestinal lamina prorpia lymphocytes in non-human primates both in normal animals and in animals having intestinal inflammation. Initially, methods were developed to isolate lymphocytes from the intestinal lamina propria, characterize them with a variety of monoclonal antibodies, and characerize their immunoregulatory and cytotoxic function.

In animals having intestinal inflammation caused by the human pathogen Lymphogranuloma venereum (LGV) we found that isolated lymphocytes were phenotypically and functionally predominantly helper T cells. Lymphocytes with the phenotype of cytolytic T cells were present, but natural killer cells were rare.

These results are comparable to studies of human intestinal inflammation in diseases such as Crohn's disease. This animal model system will be used to investigate mechanisms of regulation of intestinal inflammation and the effect of pharmacologic agents.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00447-01 LCI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
C1q: Its Biosynthesis and Biological Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Andrea Tenner, Ph.D. | Senior Staff Fellow | LCI/NIAID |
| Others: | David Bobak, M.D. | Medical Staff Fellow | LCI/NIAID |
| | Alejandro Malbran, M.D. | Fogarty Fellow | LCI/NIAID |
| | Julian Ambrus, M.D. | Medical Staff Fellow | LIR/NIAID |
| | Eric Brown, M.D. | Senior Investigator | LCI/NIAID |
| | John Bohnsack, M.D. | Medical Staff Fellow | LCI/NIAID |
| | George Martin, Ph.D. | Laboratory Chief | LDBA/NIDR |
| | Hynda Kleinman, Ph.D. | Senior Investigator | LDBA/NIDR |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Clinical Investigation

SECTION
Clinical Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.8 | 1.8 | |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The most well characterized function of C1q, a subunit of the first component of complement, C1, is its recognition of foreign material or immune complexes and subsequent initiation of the classical complement pathway ultimately leading to the lysis neutralization or clearance of the potentially detrimental agent. C1q also plays a role in the inhibition of precipitation of immune complexes. Our previous work has demonstrated that C1q binds to specific surface receptors on human peripheral blood B cells, monocytes and polymorphonuclear leukocytes. The current research program is directed toward the elucidation of the physiologic role of this interaction of C1q with its cellular receptors and with other molecules of potentially physiologic relevance. Thus the program has three main areas of focus. First, investigations of both the regulation of expression of the cellular receptor and its function have been initiated. We have determined both the number of C1q binding sites per cell and the affinity of C1q for its receptor after incubation of neutrophils and monocytes with the activation peptide f-met-leu-phe or with phorbol dibutrate (PDBu). Similarly, we have begun to investigate B cells at various stages of activation and differentiation by extending our studies to B cells derived from tonsils and spleen and further separated into subsets defined by size (i.e., the small, non-proliferating cells and the large, activated cells). Parallel functional studies suggest that C1q may affect the extent of differentiation of large cells into immunoglobulin secreting cells. Second, a specific interaction between C1q and laminin, a major macromolecular component of basement membranes, was demonstrated suggesting a possible role for C1q in the deposition of immune complexes in basement membranes. Third, we are continuing an investigation of cells which synthesize C1q, specifically fibroblasts and in vitro differentiating monocytes, to ascertain the availability of functionally active C1q at extravascular sites.



PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF IMMUNOGENETICS
1985 Annual Report
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Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

RESEARCH PROGRESS

The Laboratory of Immunogenetics investigates the multigene families that are involved in control of immune function. Our research emphasizes the structure and function of the genes and their products as well as mechanisms for regulation of these genes. Recent investigations have utilized the major histocompatibility complex, the T cell antigen receptors and the immunoglobulin gene complexes of several different species. A wide range of techniques at the molecular, serological and functional level are used in the investigations.

STUDIES OF THE HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX

The major histocompatibility complex (MHC) is under investigation by several different groups within the laboratory using a variety of molecular techniques. These studies include direct molecular analysis of the gene structure within this complex, genetic mapping of genes within the complex, structural studies of variant antigens and control of expression of the genes within the families.

Human class II genes. The genes located within the D-region of the major histocompatibility complex include those genes that encode immune responsiveness and susceptibilities to various disease conditions have been mapped to this region. These genes encode $\alpha$ and $\beta$ chains of the HLA-DR, -DQ, and -DP class II antigens. Studies have been underway (Long) to determine how many class II genes are within this complex, how many are expressed as protein, and what functions are mediated by the gene products. In these studies a cDNA library was constructed from mRNA of a human B cell line having a single HLA haplotype. Clones for $\alpha$ and $\beta$ chains of DP, DQ and DR antigens were isolated and sequenced. In addition, a clone for a new $\beta$ chain, designated DO, was isolated and sequenced (Tonnelle). This new gene maps to the D-region but is independent of DP$\beta$, DQ$\beta$ and DR$\beta$ genes and is subject to different regulatory mechanisms. The function of the DO$\beta$ gene is not known but studies are underway to determine its patterns of expression and eventually its function.

It has been possible to express individual class II antigens in human cell lines by DNA mediated transfer using the cloned genes obtained from the human cell line (Sekaly). These studies have demonstrated that functional DR1 antigens can be recognized on transfected cells by cytotoxic T clones specific for measle virus when the cells are infected with the virus. These transfection experiments also have demonstrated that it is possible to express class II antigens on cell surface in the absence of the invariant chain of the class II gene complex.

Genetic studies of the human MHC. Cloned DNA or cDNA corresponding to HLA class I, class II and class III genes have been used as probes in Southern blot analysis of DNA samples from members of eight different

families (Robinson). Restriction fragment length polymorphisms were observed and could be assigned to haplotypes in the families. Each of the families was known to include at least one individual who inherited a recombinant HLA haplotype. It has been possible to define the location of the crossover event in each recombinant haplotype and furthermore to localize the DOβ gene within the HLA complex.

More recent studies of the human families have utilized probes for the T cell receptor α and β chain genes. Polymorphic restriction fragments were observed with a probe corresponding to the $T_\beta$ gene and this was shown to segregate in six of the eight families. Haplotype assignments could be made on the basis of the polymorphism and these correlated in some instances with polymorphisms of the variable region gene segments. Polymorphism in the T cell receptor α chain was also observed in V gene segments and a possible polymorphism in the 3' untranslated region of the α gene was observed. These polymorphisms will prove useful markers to facilitate linkage studies and genetic analyses of T cell function.

Human HLA-A3 antigen variants. Earlier studies have shown that small variations in the structure of the HLA-A3 molecule could lead to differences in recognition by cytolytic T cells (CTL) of cells infected with the influenza virus. It has recently been shown in transfection experiments that mouse L cells transfected with the HLA-A3 gene and infected with the influenza virus can be recognized by specific human CTL (Cowan). These results indicate that the accessory molecules on the target cell thought to be necessary for CTL recognition in killing may be supplied by the mouse cell. Alternatively, there is no need for accessory targets and only the class I antigen and the influenza protein are necessary for recognition. Additional collaborative studies with other groups involve the structure of human T cell specific molecules T3 and T8. Viral proteins important to immune function from T cell leukemia virus and Herpes simplex virus are being investigated (Coligan).

## IMMUNOGENETIC STUDIES IN ANIMAL MODELS

Rabbit MHC genes and antigens. Studies on animal models have included studies of the rabbit MHC and of the hamster class I genes with specific emphasis on how the expression of these genes is altered by infection with adenovirus. Additional studies concern the secreted form of the class I antigen in the mouse and genes of the T cell antigen receptor complex in the rabbit.

Rabbit MHC genes and products. Studies on the rabbit MHC genes have used the cell line RL-5 derived from the inbred B/J rabbit strain by transformation with the virus *Herpes ateles*. RL-5, is by all criteria examined, a T lymphoid cell. Studies on class I antigens expressed by this cell line have identified four distinct cDNA transcripts (Marche). Complete structural data are available for these and for a genomic clone, 19-1, corresponding to the expressed antigen. A second genomic clone is available for the cDNA clone pR-11 and this includes a flaw in the transmembrane region that may preclude expression. Transfection of human Hela cells by 19-1 has given transfectants that express this rabbit product. A monoclonal antibody prepared against a peptide derived from the sequence of the 19-1 gene specifically recognized the transfected

product on the Hela cell and precipitated a molecule of the molecular weight appropriate for a class I antigen (A. LeGuern). Probes derived from the four cDNA clones obtained from RL-5 and synthetic oligonucleotides based on their sequences have been used to detect corresponding genes in genomic libraries and on Southern blots (Rebiere). Present studies attempt to classify the class I genes in terms of their expression in various tissues.

Rabbit class II genes. Studies of class II genes have begun using probes for HLA-DP, -DQ and -DR $\alpha$ genes from the human (C. LeGuern). These probes were used to search genomic and cDNA libraries from the RL-5 T cell line. To date, five distinct $\alpha$ clones have been identified based on distinct mapping patterns and on hybridization studies using the human probes as well as newly derived rabbit class II probes. Rabbit genes corresponding to HLA-DR, -DP and -DQ $\alpha$ have been identified along with a fifth $\alpha$ gene designated RLA-DN. The latter clone does not correspond to any previously reported human class II gene. Structural studies of the RLA $\alpha$ genes are underway and preliminary data suggest that the analogs of HLA-DQ and DR have high homology to the human $\alpha$ genes. Expression studies of the class II genes will continue in parallel with those of the class I genes as the probes become available.

In addition to studies of the expression of the MHC genes in normal cells and tissue, cell lines will be prepared by transformation by various oncogenic DNA viruses (Kulaga). A class II positive cell line has recently been derived from a spontaneous rabbit breast adenocarcinoma. This tumor line was shown to produce large amounts of class II antigens on the surface by immunofluorescent assays. Presently the RNA transcripts from the tumor line and from the virally transformed cell lines are being studied to determine how much and what type of class II $\alpha$ genes are being expressed.

Studies of murine histocompatibility genes. In the mouse, studies revealed a family of $L^d$-like molecules representing several different haplotypes and suggest that many D-region molecules have evolved from an $L^d$-like primordial gene (Lillehoj). The fact that the H-2 haplotype Q10 class I gene encodes a soluble molecule was established by showing that L cells transform with the gene secreted the predicted product (Lew).

Studies of class II murine antigens localize structural variations that may be involved in regulatory processes. These differences were localized to the $\alpha 1$ and $\beta 1$ domains of the respective chains of the I-A molecule.

Rabbit T cell antigen receptors. Genes encoding the T cell antigen receptor have been studied using the rabbit T cell line RL-5. Cloned cDNA corresponding to both $\alpha$ and $\beta$ chains have been obtained and a sequence of a full-length $\beta$ clone has been determined (Marche). A second cDNA clone of approximately 1 kb from the RL-5 line is presently understudy. In addition, a genomic configuration of the $\beta$ genes in RL-5 is understudy. It has been found that the structure of the $\beta$ locus is similar to that observed for the human and mouse genes. A restriction fragment length polymorphism of the $\beta$ genes was observed using the enzyme EcoRI on Southern blot analysis. Family studies using this RFLP have determined that the $\beta$ genes are autosomal, they are not linked to the

heavy chain gene complex and a possible linkage to the κ light chain gene complex has been detected (Kindt). It will be necessary to carry out further family studies to verify this linkage. Structural studies of the rabbit β chain gene have revealed 75% protein homology to mouse and human and about 80% nucleic acid homology. The V region, by contrast, has only 14-23% homology to reported human and murine sequences and may represent a member of a family not yet reported for either of these species.

Affect of viral transformation on class I expression. The hamster is being used as a model system to determine the difference in expression patterns of class I major histocompatibility antigens under the influence of transformation by adenovirus 2, adenovirus 12, polyoma and SV40 (Sogn). These studies indicate that the adenovirus 12 transformants have greatly reduced levels of mRNA encoding class I MHC antigens compared to the other lines, whereas, cell surface expression of these antigens does not appear to be diminished in adenovirus 12 transformed cells. Heterogeneity of class I antigens in the adenovirus 2 transformants has also been found. Attempts are being made to extend the results concerning adenovirus to other more well-characterized class I gene systems. Kidney cells from rabbits are being transformed with adenovirus 2 and adenovirus 12 to determine their effect on expression of class I MHC antigens in this system.

## CONTROL OF GENE EXPRESSION

Studies have been underway in several systems to explore gene regulation in the immune system (Max). In a project investigating the κ immunoglobulin genes of rabbit it was shown that all laboratory rabbits harbor two sequences hybridizing to κ probes in Southern blots. These genes were named κ1 for the nominal or expressed gene and the unexpressed gene was designated κ2. Nucleotide sequence analyses have established that the J region cluster of the κ1b5 gene resembles that of the κ1b4 in having only a single functional J gene segment within a cluster of five J-like sequences (Esworthy). Experiments in progress are aimed at analyzing the potential relationship between transcriptional regulatory sequences in the J-C intron and the relative *in vivo* expression of the different rabbit κ genes.

The chromosomal state of a known regulatory region, the enhancer of the human κ immunoglobulin gene, is being studied using genomic gene blotting techniques. Genomic sequencing methods have been understudy for some time and now have achieved a degree of sensitivity that should allow comparison of the effects of the different chromosomal proteins in cells that express and do not express the immunoglobulin genes (Gimble). It has been demonstrated in B cells that the enhancer region is unusually sensitive to certain restriction endonucleases and further studies will search for effects of chromosomal proteins in this region.

Control of the expression of the human J chain gene in B cell development has been studied (Max). The J chain gene has been cloned and the sequence of all exons and some flanking regions has been determined. Using probes from the cloned gene, J chain gene expression and gene methylation was examined in several pre-B and B cell lines. A potentially regulatory sequence 5' of the J chain gene is being

investigated using chloramphenicol acetyl transferase (CAT) transient expression system.

Annual Report
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## ADMINISTRATIVE REPORT

During the period covered by this report there has been a turnover of several positions at the postdoctoral and expert level within the Laboratory of Immunogenetics. Dr. R. Steve Esworthy left the laboratory to assume a position at City of Hope in Duarte, California. Dr. Cecile Tonnelle will be returning to her position at CNRS-INSERM Institut in Marseille. Dr. Christian LeGuern will resume his position as Professor at the University of Paris and staff member of the Institut Pasteur. Dr. Annie LeGuern will return to her position at the Institut Pasteur. Dr. Fathia Mami from the Institut Pasteur has joined the Immunogenetics Research Section of the laboratory and Dr. Margherita Bagnato from the University of Milan has joined the laboratory in the Immunobiology Section. Dr. Nazma Jahan joined the Immunogenetics Research Section in the group headed by Dr. Max. Dr. Christophe Girardet from the Ludwig Institute of Cancer Research spent three months in the laboratory of Dr. Eric Long as a Guest Worker. Dr. Sandra Rosen-Bronson from Georgetown University has joined Dr. Eric Long's laboratory as a Staff Fellow.

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## HONORS AND AWARDS

Dr. Kindt has presented invited lectures concerning laboratory results at the University of Paris, at the Institut Pasteur and at the University of Uppsala in Sweden. In addition he has presented lectures at the University of Michigan, West Virginia University School of Medicine, Amherst College, Sloan Kettering Institute, Georgetown University and Emory University. He was a guest lecturer at the Cold Spring Harbor Institute and at a mini-symposium at the FASEB meeting. Dr. Kindt continues to serve as Section Editor of the Journal of Immunology, is on the Editorial Board of the Journal of Biological Chemistry, the Journal of Experimental Medicine, and Contemporary Topics in Immunology. He serves on the Study Section of the American Cancer Society and has recently been invited to serve on the Study Section of the Multiple Sclerosis Society. In addition, he has served as an ad hoc reviewer for grants and projects for the National Cancer Institute. This year Dr. Kindt was given the Assistant Secretary of Health Award for exceptional achievement. Dr. Mary Ann Robinson received the Sandoz-Amos Award this spring. She has given presentations at the ASHI Meeting and served on the Education Committee and has recently been elected to the Program Committee of this Society. Dr. John Coligan has given invited lectures at the Dana-Farber Cancer Center, New York Medical College, Columbia University and Hospital for Joint Diseases. He has made presentations at the Miami Winter Symposium and at the FASEB Meeting. This year he was given the NIH Director's Award. Dr. Coligan has been reelected to the Editorial Board of the Journal of Immunology and continues to serve as Editor for Survey of Immunologic Research. Dr. Edward Max has received the PHS Medal and has been invited to serve as Editor for the Journal of Immunology. Dr. Max presented data from his laboratory at the FASEB meeting and has given invited seminars at several different universities. Dr. John Sogn was an invited speaker at the International Workshop on Pentraxins and has been invited to serve as Associate Editor for the Journal of Immunology. He has been a lecturer in a series of workshops on hybridomas held at various locations throughout the country. Dr. Eric Long presented data from his laboratory at Howard University, Johns Hopkins, the Imperial Cancer Research Fund in London and the University of Geneva Medical School this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00166-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Rabbit MHC antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Thomas J. Kindt | Chief | LIG/NIAID |
| OTHER: | Patrice N. Marche | Visiting Fellow | LIG/NIAID |
| | Marie Christine Rebiere | Visiting Fellow | LIG/NIAID |
| | Annie LeGuern | Guest Worker | LIG/NIAID |
| | Christian LeGuern | Expert | LIG/NIAID |
| | Edward E. Max | Commissioned Officer | LIG/NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.8 | 2.7 | 1.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the rabbit class I antigens expressed by the rabbit T cell line RL-5 have identified four distinct cDNA transcripts. Complete structural data are available for these and for a genomic clone, 19-1, corresponding to the one expressed gene (pR9) of the rabbit T cell line. Transfection of human HELA cells with 19-1 gave cells expressing the rabbit product. A second genomic sequence is available for the clone, designated pR11, and this indicates a flaw in the transmembrane region that may preclude its expression. Probes derived from the four cDNA clones and synthetic oligonucleotide probes based on their sequences were used to detect genes corresponding to them in genomic libraries and on Southern blots. These probes will be used to classify the rabbit class I genes in terms of expression in various tissues. Studies of rabbit class II antigens have begun using probes for HLA-DP, -DQ and -DR α genes. These probes have been used to search genomic and cDNA libraries from the RL-5 T cell line. To date, five distinct α clones have been identified based on distinct mapping patterns and on hybridization studies using the human probe and the newly derived rabbit class II probes. Rabbit genes corresponding to HLA-DR, -DP1, -DP2 and -DQ α have been identified along with a fifth α gene designated RLA-DN that does not correspond to any previously reported class II gene. Structural studies of the RLA α genes are underway and preliminary data suggest that they have a high degree of homology to the human and α genes. Expression studies of the class II genes will continue in parallel with those of the class I genes as the probes become available.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00168-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Surface Markers of Rabbit Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Thomas J. Kindt | Chief | LIG/NIAID |
| OTHER: | Patrice N. Marche | Visiting Fellow | LIG/NIAID |
| | Fathia Mami | Visiting Fellow | LIG/NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland, 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.4 | 1.8 | 1.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes encoding the T cell antigen receptor have been studied using the rabbit T cell line RL-5. Cloned cDNA corresponding to both alpha and beta chains of the T cell receptor has been obtained. The sequence of a full-length β chain clone of 1.3 kb in length which includes the V, D, J and C region has been determined. A second cDNA clone of approximately 1 kb from RL-5 is presently under study. Two distinct clones corresponding to the α genes were identified in the RL-5 cDNA library using a murine α chain probe and analysis is underway. A restriction fragment length polymorphism (RFLP) of the β chain genes was observed using the enzyme EcoRI on Southern blot analysis. Family studies using this RFLP have determined that the β genes are autosomal, that they have no linkage to the heavy chain gene complex and that there is a possible linkage to the κ light chain gene complex. Studies of the β genes in a genomic library have revealed the presence of two $C_\beta$ genes located about 7 kb apart. The full-length cDNA transcript corresponds to the 3' $C_\beta$ gene and therefore it is the homolog of $C_\beta 2$ in the mouse and human. Structural comparisons of rabbit β chain genes reveal 75% protein homology to mouse and human and about 80% nucleic acid homology. The rabbit V region sequence, by contrast, has only 14-23% homology to reported human and murine sequences.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00169-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of Murine and Human Transplantation Antigens and Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John E. Coligan | Senior Investigator | LIG/NIAID |
| Other: | Elliot Cowan | Staff Fellow | LIG/NIAID |
| | Erik Lillehoj | Staff Fellow | LIG/NIAID |
| | Andrew Lew | Visiting Fellow | LIG/NIAID |

COOPERATING UNITS *(if any)*

W. Biddison, NINCDS, NIH; T. Hansen, Washington University at St. Louis, Jim Devlin, Biogen Research Corp, Boston, MA, E. Wakeland, University of Florida, Gainesville, FL.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.8 | 2.4 | 1.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human and murine major histocompatibility complex encoded class I molecules that are integrally involved in the recognition of virally infected cells by cytotoxic T lymphocytes (CTL) are isolated and their primary structure analyzed. The goal of these studies is to gain an understanding in molecular terms of their function and antigenic properties, as well as to obtain knowledge of their evolutionary relationships. In the case of human class I molecules, an HLA-A3 variant (E1) that is altered in its recognition by HLA-A3-influenza specific CTL has been analyzed. The altered functional properties of this variant were attributed to two amino acid substitutions that resulted from 3 base changes in the E1 gene. Analysis of the E1-gene product in murine fibroblasts after transfection demonstrated that human CTL can recognize human class I molecules on targets that do not express any other human gene product, and suggested that the effector T cell molecules T8 and LFA-1 are functionally involved in this recognition process. In the mouse studies have revealed a family of $L^d$-like molecules representing several distinct haplotypes and suggest that many D-region molecules have evolved from an $L^d$-like primordial gene. The fact that the $H-2^b$ haplotype Q10 class I gene encodes a soluble molecule was established by showing that L cells transformed with the gene secrete the predicted product. Class II molecules through their interaction with antigens are important for regulation of the antibody response by T-helper cells. Structural variations in $IA^k$ molecules involved in this regulatory process have been localized to the α1 and β1 domains.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00170-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Human Histocompatibility Antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Eric O. Long | Visiting Scientist | LIG/NIAID |
| OTHER: | Cecile Tonnelle | Visiting Fellow | LIG/NIAID |
| | Rafick Sekaly | Visiting Fellow | LIG/NIAID |
| | Sandra Rosen-Bronson | Staff Fellow | LIG/NIAID |

COOPERATING UNITS *(if any)*

David Eckels, The Blood Center of Southeastern Wisconsin; Steven Jacobson, NINCDS; Robert DeMars, University of Wisconsin

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.9 | 2.9 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The human immune response genes are located in the D region of the major histocompatibility complex. They encode the α and β chains of the HLA-DR, -DQ and -DP class II antigens. The aim of this project is to define by a molecular approach how many functional class II genes exist and to express individual class II antigens in human cell lines by DNA-mediated gene transfer in order to analyze the interaction between class II antigens and T lymphocytes. A cDNA library in a eukaryotic expression vector was constructed from mRNA of a human B-cell line having a single HLA haplotype. Expressible clones for the α and the β chains of DP, DQ and DR antigens were isolated. In addition, a clone for a new β chain, designated DO, was isolated and sequenced. The DOβ gene maps in the D region but has evolved independently from the DPβ, DQβ and DRβ genes and is subject to different regulatory mechanisms. The atypical evolution and expression of DOβ suggest that it may be part of a new class II antigen with a distinct function. Human fibroblast lines transfected with the DR1 α and β genes have been obtained which express DR antigens at the cell surface. These DR1 antigens are functional; the transfected cells are recognized by DR1-restricted cytotoxic T cell clones specific for measles virus when the cells are infected with measles virus. This system can be used to define the elements important for antigen-recognition by T lymphocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00171-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John A. Sogn | Senior Investigator | LIG/NIAID |
| OTHER: | Fathia Mami | Visiting Fellow | LIG/NIAID |
| | Henrietta Kulaga | Staff Fellow | LIG/NIAID |

COOPERATING UNITS *(if any)*

J. Coe, Rocky Mountain Laboratory, NIAID; A. M. Lewis, LIP, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.7 | 1.2 | 0.5 |

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☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The influence of adenovirus transformation on expression of class I major histocompatibility antigens in the hamster is under investigation. Hamster embryo cells transformed with adenovirus 2, adenovirus 12, polyoma and SV40 are being examined, along with untransformed cells as a control. Results to date indicate that, at the RNA level, adenovirus 12 transformants have greatly reduced levels of mRNA encoding class I MHC antigens, a result in accord with findings for rat and mouse cells. However, cell surface expression of these antigens is not diminished to the same extent, so adenovirus 12 transformed cells express levels of class I antigens at the surface very nearly equivalent to those of most adenovirus 2-transformed cells or untransformed cells. A heterogeneity of class I antigen levels in the adenovirus 2 transformants has also been found. Continuing study of hamster female protein (FP) has focused on some aspects of the protein structure which may account for some of the unusual properties of FP in serum. The nature of its carbohydrate moiety has been defined and the arrangement of its sulfhydryl groups and disulfide bonds is under study. Free sulfhydryl groups have been found but their source is as yet known. Rabbit immunoglobulin latent allotypes have been further defined by protein sequence.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00173-08 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Edward E. Max | Commissioned Officer | LIG/NIAID |
| OTHER: | Jeffrey Gimble | Medical Staff Fellow | LIG/NIAID |
| | Steve Esworthy | Staff Fellow | LIG/NIAID |
| | Nazma Jahan | Visiting Fellow | LIG/NIAID |

COOPERATING UNITS (if any)

Stanley Korsmeyer, Metabolism Branch, NCI, Wesley O. McBride, Laboratory of Biochemistry, NCI

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetic Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.7 | 2.7 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The regulation of gene expression is being studied in three systems involving the immune system. We previously showed that all laboratory rabbits in our study harbored two sequences hybridizing to κ immunoglobulin gene probes in Southern blots: the gene for the "nominal" expressed κ1 chain and an unexpressed gene encoding a κ2 isotype light chain. We have established that the J region cluster of the κ1b5 gene resembles the κ1b4 cluster in containing only a single apparently functional J gene segment within a cluster of five J-like sequences. Experiments in progress are aimed at analyzing the potential relationship between transcriptional regulatory sequences in the J-C intron and the relative <u>in</u> <u>vivo</u> expression of different rabbit κ genes.

In a second project we are attempting to examine the chromosomal state of a known regulatory region (enhancer) of the human κ immunoglobulin gene using genomic gene blotting technology, including genomic sequencing methods which have achieved the degree of sensitivity to allow comparison of the effects of different chromosomal proteins (e.g., in B versus T cells) on the accessibility of the κ enhancer region to DNA-modifying reagents (footprint analysis). This region is, in a B cell, unusually sensitive to certain restriction endonucleases.

In a third project we have cloned the complete J chain gene and determined the nucleotide sequence of all the exons and some flanking regions. Using probes from the cloned gene we have examined J chain gene expression and gene methylation in several pre-B and B cell lines. Currently we are attempting to assess the potential regulatory function of a sequence 5' of the gene using a chloramphenicol acetyl transferase (CAT) transient expression system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00180-07 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Properties of Transformed Rabbit Cell Lines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John A. Sogn | Research Chemist | LIG/NIAID |
| OTHER: | Henrietta Kulaga | Staff Fellow | LIG/NIAID |
| | Christian LeGuern | Visiting Scientist | LIG/NIAID |

COOPERATING UNITS *(if any)*

G. Marti, Hematology Branch, Clinical Center, NIH.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.3 | 0.7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major focus of this research has been the pattern of expression of rabbit class II major histocompatibility antigens. The genes encoding the α chains of these antigens have been recently cloned in this laboratory and is described in another section of this report. Probes derived from the cloned rabbit class II MHC antigen genes and human class II probes have been used to examine mRNA prepared from normal rabbit tissues and a number of newly established class II-positive in vitro propagated cell lines. The first objective is to determine which genes are normally expressed and in what cell types the expression occurs. Using the DCα probe, mRNA from spleen and appendix were strongly positive, as expected for rich sources of B lymphocytes. Lymph node was positive at a lower level as were lung and testes. The result with testes is undergoing closer examination to determine the cellular source of the signal. Class II antigen-positive cell lines have been prepared by viral transformation of rabbit cells with several oncogenic DNA viruses. In addition, a class II-positive cell line has been derived from a spontaneous rabbit breast adenocarcinoma. These cell lines have been shown to contain mRNA transcripts reactive with human class II probes and are being probed now to determine their expression of each of the cloned rabbit class II α chains.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00352-03 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Cell Surface Molecules Important for Immune Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John E. Coligan | Senior Investigator | LIG/NIAID |
| OTHER: | Andrew Lew | Visiting Fellow | LIG/NIAID |

COOPERATING UNITS *(if any)*

Cox Terhorst, Dana-Farber Cancer Institute, T.-H. Lee, Harvard School of Public Health; R. Nairn, University of Michigan; M. Rechler, NIADDKD

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.2 | 1.6 | 1.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple molecules exist on the surface of lymphocytes which are important for the development of the immune response. A major goal of these studies is to identify and structurally characterize these membrane-bound molecules, especially those present on cytotoxic T lymphocytes. Concordantly, it is important to have an understanding of the molecules encoded by infectious agents which are recognized by the immune system. Thus, the nature of the antigens in several viruses posing serious health problems is being investigated. Project areas include: (1) studies on the gene and protein structures of the human T cell molecules T3 and T8; (2) characterization of the molecules of antigenic importance in human T cell leukemia viruses (HTLV) and the AIDS virus; and (3) studies on antigenic variation in Herpes simplex virus type 1 (HSV-1) glycoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00389-02 LIG

PERIOD COVERED

October 1, 1984 to September, 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genotype Analyses in HLA Recombinant Human Families

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Mary Ann Robinson | Senior Staff Fellow | LIG/NIAID |
| OTHER: | Thomas J. Kindt | Chief | LIG/NIAID |

COOPERATING UNITS *(if any)*

A. Johnson, Georgetown University and Bernard Amos, Duke University

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.4 | .7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes known to play important roles in a variety of immune processes have been genetically analyzed in several human families. Cloned DNA or cDNA corresponding to HLA class I, class II (DRα, DRβ, DQα, DQβ, DOα, DOβ and DPβ) and class III (C4) genes and to T cell receptor α and β chain genes were used as probes in Southern blotting analysis of DNA samples from the members of eight families. Restriction fragment length polymorphisms were observed with certain enzymes and could be assigned to haplotypes in the families. Each of the families was known to include at least one individual who inherited a recombinant HLA haplotype. The present studies have made it possible to more precisely define the location of the crossover event in each recombinant haplotype and to localize DOβ a new class II β chain gene within the HLA complex. Polymorphic restriction fragments hybridizing with a probe corresponding to the constant region of the T cell receptor β chain gene were observed to segregate in six of the eight families. Haplotype assignments could be made on the basis of these polymorphisms as well as with polymorphic fragments that hybridize to probes corresponding to variable (V) gene segments. Polymorphism in T cell receptor α chain genes was observed to be located in V gene segments or the 3' untranslated region. These polymorphisms will provide useful markers that will facilitate linkage studies and genetic analyses of T cell function.



PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF IMMUNOLOGY
1985 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1984 through September 30, 1985

## Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It had made rapid progress through the use of three new technologies which are creating a revolution in immunologic science. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and major improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important advances in the efforts to more precisely regulate the normal and the disordered immune response.

## Characterization of the XLR Gene Family

Laboratory of Immunology scientists have identified a set of genes found on the X chromosome of mice which constitute a gene family designated XLR. Some members of this family are expressed in B and T lymphocyte tumors, but expression has not yet been detected in non-lymphoid cells. In lymphomas of the B cell lineage, expression of XLR genes appears correlated with developmental state. Abelson murine leukemia virus-induced pre-B cell lines do not express XLR mRNA, nor do B lymphomas of very immature characteristics such as WEHI-231. Among "mature" B lymphomas, expression of XLR mRNA is correlated with preparation to secrete immunoglobulin as detected by hypomethylation or actual expression of J chain genes. Plasmacytomas from normal mice all express XLR mRNA. However, presecretory cells and secretory cells appear to differ in that the former express mRNA of only a single size whereas the latter have mRNA of two distinct sizes. Analysis of cDNA clones indicates that the presecretory cells express an XLR mRNA also expressed in secretory cells. Nucleotide sequencing of one XLR cDNA indicates an open reading frame which can code for a protein of $M_r$ of 24,000 which is hydrophilic and acidic but which lacks both a leader sequence and a transmembrane sequence, suggesting it is neither a secreted or a membrane protein.

Analysis of congenic mice indicates a strong linkage between XLR genes and the <u>xid</u> gene, which is associated with a B lymphocyte immunodeficiency characterized by unresponsiveness to class II antigens. Further evidence of association between XLR and xid is that plasmacytomas generated in NZB.xid mice fail to express XLR genes in their mRNA whereas as NZB (and BALB/c) plasmacytomas do express XLR as described above. The XLR gene family promises to be of great importance in the regulation of lymphocyte development.

Efforts to examine this idea by gene cloning, transfection, and production of transgenic mice are in progress or planned (Cohen and Paul, LI/NIAID/ Siegel and Steinberg, A&R/NIADDK; Davis, Stanford University).

Structure-Function Analysis of Class I Major Histocompatibility Complex (MHC) Molecules

Class I MHC molecules consist of an ~45,000 $M_r$ glycoprotein chain coexpressed with β-2 microglobulin (β-2M) on the surface of most cell types. The cell surface expression of the class I glycoprotein chain appears to depend upon its pairing with β-2M and the third external domain (C2) of the membrane glycoprotein appears responsible for the binding of β-2M. Laboratory of Immunology scientists have undertaken to examine the structural basis of these observations utilizing chimeric and truncated class I MHC genes and and DNA mediated gene transfer experiments. Truncated H-2$D^d$ and H-2$L^d$ class I genes, coding for only the C-2 and more carboxy-terminal domains, are expressed on the cell membrane and do pair with β-2M, confirming less direct results on the critical role of C2 in this phenomenon. Moreover, cytotoxic T cells capable of specificially lysing C2 transfectants can be obtained suggesting that structures on this domain can elicit specific T cell responses. Chimeric genes, in which 5' exons from class II β chain genes are ligated onto constructions containing the C2 and more 3' exons of H-2$D^d$ have also been prepared and introduced into L cells. These transfectants express the chimeric molecule on their membrane and cytotoxic T cells specific for allogeneic class II molecules recognize L cells expressing these chimeric molecules. Strikingly, anti-L3T4 antibodies block this killing establishing that the role of L3T4 in T cell antigen-recognition does not require the presence of either class II α chain or the $\beta_2$ domain. This result throws serious doubt on the concept that L3T4 functions as an auxilliary molecule to recognize constant regions of membrane class II molecules. These experiments also led to the detection of an alternatively spliced form of H-2$D^d$ in which exon VII, containing a potential phosphorylation site, has been deleted. The biologic significance of this alternative form of H-2$D^d$ has not yet been determined (McCluskey and Margulies, LI/NIAID; Maloy and Coligan, LIG/NIAID; Golding, Singer & Bluestone, IB/NCI).

Assembly and Expression of Class II Major Histocompatibility Complex (MHC) Molecules

Class II MHC molecules are composed of two chains, α and β, which are both encoded by genes in the I-region of the MHC. Rules concerning assembly, pairing, and expression of class II α and β chains had been largely determined by conventional breeding experiments, which, of necessity provided very indirect evidence. Laboratory of Immunology scientists have undertaken DNA-mediated gene transfer experiments to allow the direct assessment of the mechanisms determining assembly of class II molecules. L cells were transfected with haplotype matched or mismatched $A_\beta$ and $A_\alpha$ genes. Cotransfection with haplotype mismatched pairs (e.g. $A^k_\beta A^d_\alpha$) has led to

variable levels of expression ranging from undetectable in the case of $A^d_\beta A^k_\alpha$ to moderate in the case of $A^b_\beta A^k_\alpha$; these levels have never been as high as haplotype matched pairs. To determine the portion of the $A_\beta$ molecule involved in this preferential pairing, recombinant $A_\beta$ genes have been prepared. Use of these genes have yielded results indicating that the $\beta_1$ domain was the critical region for assembly. Furthermore, cotransfection studies also indicate that the formerly held concept the α and β chains from the I-A and I-E regions do not pair with one another is incorrect. When $A^d_\beta$ and $E^k_\alpha$ genes are transfected into L cells, these chains are assembled and expressed on the membrane.

These results indicate that a) transcomplementation may contribute less to overall polymorphism of class II MHC molecules than initially believed; b) upregulation of hybrid (or "cross-region") class II molecules from undetectable to stimulatory levels by agents such as interferon γ or B cell stimulatory factor-1 may lead to auto-immune responses to these formerly cryptic molecules; c) the I region of the MHC has evolved to keep $A_\beta$ and $A_\alpha$ pairs in strong linkage disequilibrium to avoid the diminution in class II molecule expression which might be anticipated in recombinants (Braunstein, Sant & Germain, LI/NIAID).

Structure-Function Analysis of Class II Major Histocompatibility Complex (MHC) Molecules

Class II MHC molecules are heterodimers consisting of α and β chains which play a critical role in antigen-recognition by $L3T4^+$ T cells. They appear to form complexes with antigenic peptides which are corecognized by the T cell receptor for antigen. Furthermore, the failure of some class II molecules to be capable of being co-recognized with certain antigens is responsible for immune response (Ir) gene determined unresponsiveness to those antigens. Spontaneously occurring or selected mutants effecting the β chain of the I-A class II molecule show the loss of ability to present antigen to some T cell hybridomas to which the wild type cell presents antigen normally. DNA mediated gene transfer experiments indicate that in such mutants the β chain is responsible for mutant function. Moreover, construction of chimeric β chain genes possessing leader and $\beta_1$ exons from the mutant and more 3' exons from wild type donors indicate that the functional abnormality of the mutant is determined by the N-terminal domain of the β chain. One of these mutants (bm12) had previously been shown to have nucleotide substitutions leading to amino acid changes at positions 67, 70, and 71 of the β chain. Nucleotide sequencing of a mutant selected in the Laboratory of Immunology indicates a single nucleotide change resulting in the replacement of glutamic acid with lysine at position 67. The concept of the critical role of the $\beta_1$ domain has further

been established by preparing exon shuffled genes involving the $\beta_1$ and $\beta_2$ domains and, in some cases, within the $\beta_1$ domain itself. In addition, site directed mutants have been prepared and transferred to L cells. These results, taken together, indicate a major role for the third hypervariable region of the $\beta_1$ domain in T cell recognition (or corecognition) of class II molecules, with single amino acid changes in this region having striking effects on individual T cell responses. Additional sites at a distance on the linear, although not necessarily on the three dimensional, map of the $A_\beta$ chain are also involved in function of class II molecules in antigen-recognition (Lechler, Ronchese, Braunstein, Brown, Paul & Germain, LI/NIAID).

Molecular Genetic Analysis of the Ontogeny of T Cell Receptor Expression

The T cell receptor for antigen is a heterodimer consisting of dissulfide linked α and β chains, both of which contain variable and constant regions. Mature T cells which express these receptors are derived from thymocytes which in turn derive from hematopoietic cells which seed the thymus. A subset of thymocytes which lack the Lyt2 and L3T4 lineage markers and express small amounts of Lyl have been shown, by repopulation studies, to represent immature thymocytes. Northern analysis of RNA from these "dull-Lyl" cells indicates expression of β chain mRNA but little or no α chain mRNA, while more mature thymocytes express mRNA for both α and β chains. Hybridomas were prepared from "dull Lyl" thymocytes. Some of these had both α and β chain genes in germ line configuration while others had rearranged β chain genes. These results suggest that in the course of T cell development, β chain rearrangement precedes α chain rearrangement. Furthermore, they are consistent with the concept that rearrangements of β chain genes first occur for many T cells after hematopoietic precursors have entered the thymus, implying an absence of receptor expression on pre-T cells (Samelson, Germain & Schwartz, LI/NIAID; Fowlkes, LMI/NIAID; Lindsten and Davis, Stanford University; van den Elsen and Terhorst, Harvard Medical School).

A Receptor Competition Assay for the Relative Affinity of T Cell Receptors

It is now clear that T cells co-recognize antigenic epitopes and structures on class I or class II major histocompatibility complex (MHC) molecules. Since the conjoint determinant appears to be formed only in cell membranes, and, as recently shown, in artificial lipid membranes, direct measurements of the affinity of T cell receptor-ligand interactions have not been possible. Laboratory of Immunology scientists have now developed an assay which allows the relative affinity of this interaction to be determined. It is based on the observation that in the interaction of an antigen-specific, MHC-restricted T cell clone with antigen and antigen-presenting cells, an increase in the number of responding T cells required a concommitant increase in the concentration of nominal antigen to achieve any given fraction of the maximum cellular response. However, this shift in the antigen concentration-response curve only occurred when the number of T cells was increased beyond a critical

point, designated the transition point. These results have been interpreted to indicate that as cell number (and receptor number) increase, receptors compete for available antigen. Thus, when receptor concentration is sufficient to bind a significant fraction of total antigen, free antigen concentration can only be kept constant by increasing the initial antigen concentration. The transition point obviously reflects a parameter related to the number and affinity of cellular receptors; in situations such as with T cell clones, when receptor number is a constant, the transition points for any set of ligands reflect the relative affinity of these ligands for the antigen-MHC molecule complex. This technique was applied to the cytochrome c system and was used to validate previous results which indicated that amino acid 99 was a contact site between the T cell receptor and a cytochrome c peptide. The further use of this method should be of considerable importance in analysis of interaction of mutant and wild type class II MHC molecules with antigen and T cell receptors and should aid in structure-function analysis of the critical but complex phenomenon of T cell recognition of antigen (Ashwell and Schwartz, LI/NIAID).

Phosphorylation of a T-Cell Receptor Associated Protein

T cells recognize complexes of antigen and major histocompatibility complex (MHC) molecules through a membrane glycoprotein which, like immunoglobulin, is organized into variable and constant regions. This antigen-binding element consists of disulfide-linked α and β chains and is closely associated with a set of invariant membrane proteins designated the T3 complex in human T cells. Although these proteins are not well characterized in the mouse, coprecipitation studies indicate that murine T3 analogs exist. Since the T3 complex is believed to be involved in signal transduction, Laboratory of Immunology scientists sought evidence for covalent modification of T3 analogs in the course of T cell activation. A set of antigen-specific MHC restricted T cell hybridomas were labelled with $^{32}P$ and activated with antigen and antigen-presenting cells or with concanavalin A. Immunoprecipitates were prepared with antibodies to clonotypic determinants on the T cell receptor and these were analyzed by radioautography of sodium dodecyl sulfate-polyacrylamide gel electropherograms. No phosphorylation of the receptor itself was noted but a coprecipitated 20,000 $M_r$ protein was rapidly phosphorylated after either antigen or concanavalin A activation. This protein appears to be consitutively associated with the T cell receptor and appears to be an excellent candidate to play a critical role in the cellular activation process. Efforts to determine whether this event is essential for signal transduction are now in progress (Samelson and Schwartz, LI/NIAID; Harford and Klausner, LBM/NIADDK).

## Thy 1 as a T Cell Activation Molecule

Thy 1 is a membrane glycoprotein expressed on mouse T cells and on certain other cell types, including cells in the central nervous system. Some alloantisera to Thy 1 and a monoclonal anti-Thy 1 antibody, G7, cause proliferation of normal T cells and stimulate interleukin-2 (IL-2) production by many antigen-specific T cell hybridomas. In order to gain an understanding of the role of Thy 1 in T cell activation, Laboratory of Immunology scientists have prepared a large panel of anti-Thy 1 monoclonal antibodies and have derived genomic clones of Thy 1 for DNA-mediated gene transfer experiments. Of the additional monoclonal antibodies examined, two displayed some T cell stimulatory activity in that they could induce T cell proliferation and IL-2 production, but only when used in combination and as costimulants with phorbol myristate acetate (PMA). All three of these stimulatory monoclonal antibodies caused a rapid rise in intracellular free calcium concentrate $[Ca^{++}]_i$ of T cells.

A full length genomic clone for Thy 1 was inserted into an expression vector and transferred into the human T cell tumor line Jurkat by means of spheroplast fusion. Four independent Thy-1$^+$ transfectants produced IL-2 when stimulated with PMA and monoclonal anti-Thy-1 antibodies. One of these transfectants, although reactive to anti-Thy-1, had lost reactivity to antibody to the T3 molecule, normally a potent stimulator of Jurkat cells. A Thy-1 loss variant of this line reacquired its responsiveness to T3 and to monoclonal antibodies to clonotypic determinants on Jurkat's T cell receptor. These results raise the possibility of some type of reciprocal relationship between the T3 complex and Thy 1 with the signal transduction mechanism of T cells (Gunter, Kroczek, Miller, Germain and Shevach, LI/NIAID).

## Regulation of Expression of the Receptor for Interleukin 2

The stimulation of T cells to divide is dependent on the interaction of the lymphokine interleukin-2 (IL-2) with membrane receptors for IL-2 expressed only on activated T cells. This IL-2 dependent stimulation of T cells has several important properties including the fact that IL-2 can be made by cells which have IL-2 receptors and, as shown by Laboratory of Immunology scientists, that IL-2 upregulates its own receptor. A key element in carrying out this work was the derivation of a cDNA clone that contains the entire 804 base pair coding region of the murine IL-2 receptor. The sequence of the mouse IL-2 receptor reveals regions of high homology with the human IL-2 receptor. Using this cDNA clone to analyze IL-2 receptor control in an antigen-specific T cell clone established that IL-2 itself upregulated both membrane IL-2 receptor levels and the amount of cytoplasmic mRNA for IL-2. These experiments indicate that, in normal T cell populations, initial acquisition of IL-2 receptors renders the cell sensitive to further increase in IL-2 receptor number as a result of the action of IL-2 itself and suggest that this

regulation of IL-2 receptor number is transcriptionally controlled. The information arising from this analysis should be of great importance in understanding the cell biology of T cell responses and in designing pharmacologic approaches to regulate T cell proliferation (Malek, Ashwell, Germain, Miller and Shevach, LI/NIAID; Leonard and Greene, MET/NCI).

Cellular Biochemistry of B Cell Responses to Anti-IgM Antibodies

Resting B cells cultured with anti-IgM antibodies are stimulated to enter the $G_1$ phase of the cell cycle and will synthesize DNA if B cell stimulatory factor (BSF)-1 is also present. This activation appears to result from an intracellular signalling process dependent upon crosslinkage of membrane IgM by anti-IgM antibodies. B cells display rapid increases in intracellular free calcium concentration $[Ca^{++}]_i$ in response to anti-IgM as measured by fluorescence of the Ca sensitive dye, Quin 2. Resting B cells have a $[Ca^{++}]_i$ of ∿100nM which increases to ∿200nM within minutes of addition of anti-IgM. When cultured in Ca-free medium and in the presence of the chelating agent EGTA, the increase in $[Ca^{++}]_i$ is very markedly diminished, but not abolished. This suggests that some of the calcium is mobilized from intracellular stores. In parallel with increased $[Ca^{++}]_i$ is an increase in inositol phosphates. This suggests that anti-IgM leads to activation of protein kinase C and that subsequent steps in the activation program may depend upon phosphorylation. The importance of increase in $[Ca^{++}]_i$ and protein kinase C activation in B cell responses is supported by the finding the culture of B cells with phorbol myristate acetate (PMA) and a calcium ionophore such as A23187 causes effects in B cells similar to those mediated by anti-IgM. Even more strikingly, PMA by itself is a potent inhibitor of B cell responses to anti-IgM and BSF-1 and inhibits both the increase in $[Ca^{++}]_i$ and inositol phosphates normally stimulated by anti-IgM. This suggests that some type of feedback activity of protein kinase C may limit further signalling through the receptor-ligand interaction and may allow identification of proteins which participate in regulating signal transduction (Mizuguchi and Paul, LI/NIAID; Beaven, IRLC/NHLBI).

B Cell Stimulatory Factor (BSF)-1: Production of a Monoclonal Antibody and Analysis of Function

BSF-1 was initially recognized as a costimulant of B cell responses to anti-IgM antibodies. It is a T cell derived product which can be chemically separated from interleukin-2 (IL-2). Laboratory of Immunology scientists have prepared highly purified BSF-1 by the use of trimethylsilyated control pore glass bead adsorption, reverse phase high pressure liquid chromatography (RP-HPLC), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Amino terminal amino acid sequences have been obtained from a set of 4 distinct bands migrating at $M_r$ ∿20,000 on SDS-PAGE. BSF-1 has been successfully translated from polyA$^+$ RNA in Xenopus laevis oocytes and efforts to prepare cDNA clones are underway.

RP-HPLC purified BSF-1 has been used as an immunogen to prepare a rat monoclonal anti-BSF-1 antibody. This antibody blocks BSF-1 activity at concentrations as low as 40ng/ml but has no effect on IL-1, IL-2, or IL-3 activity. Antibody conjugated columns have been used for affinity purification of BSF-1.

The availability of pure BSF-1 has allowed a reexamination of its function. Resting B cells cultured with $\sim$10U of BSF-1/ml show a small but significant increase in cell volume, a striking enhancement ($\sim$6fold) in the number of class II major histocompatibility complex (MHC) molecules on their membrane, and are prepared to enter S phase more promptly upon subsequent stimulation with receptor-specific ligands. By contrast, B cell blasts prepared with lipopolysaccharides (LPS) or anti-IgM plus BSF-1 do not enter S when cultured with BSF-1. These results indicate that BSF-1 is a powerful activation factor but has little or no activity as a growth factor. Recent evidence indicates that BSF-1 also plays a role in $IgG_1$ production by B cells cultured with LPS. The activity formerly referred to as $BCDF_\gamma$ has now been shown to be a property of BSF-1 (Ohara, Rabin, Brown and Paul, LI/NIAID; Maloy and Coligan, LIG/NIAID; Vitetta, University of Texas Southwestern Medical School).

## Molecular Basis of Kappa Chain Abnormality in Basilea Rabbits

Basilea rabbits are an apparent mutant strain of rabbits which fail to produce immunoglobulin $\kappa$ light chains of the predominant K1 isotype. However, these rabbits do produce immunoglobulin with $\kappa$ light chains of the normally rare K2 isotype and with $\lambda$ light chains. The availability of these animals presented a model system in which to evaluate mechanisms through which such critical genetic defects might arise. To examine this issue, Laboratory of Immunology scientists have prepared molecular clones for normal and Basilea $\kappa$ genes. In particular, a genomic clone of the K1b9 gene of the Basilea rabbit was isolated and sequenced. When compared to the K1b9 gene of normal rabbits, a substitution of an A for G was observed in the highly conserved AG dinucleotide of the 3' acceptor splice site, suggesting that the defect in expression of this gene arises from the inability of its RNA to be normally processed to mature, functional mRNA. These experiments thus provide a precise molecular explanation for a striking immunologic abnormality (Lamoyi, McCartney-Francis & Mage, LI/NIAID).

## Chemical Characterization of the Tumor Specific Transplantation Antigen (TSTA) of the Guinea Pig Leukemia L2C

The L2C leukemia is a transplantable leukemia of B lymphocytes. This leukemia, which arose in strain 2 guinea pigs, has a potent TSTA which can be easily demonstrated by immunization protection tests. This potency of the TSTA and the rapid fatality of the L2C leukemia in unimmunized guinea pigs make this a valuable model in which to determine the chemical nature of the TSTA. Laboratory of Immunology

scientists have now made very substantial progress in determining the nature of this TSTA. Potassium chloride extracts of L2C cells contain a molecule(s) which on immunization protects strain 2 guinea pigs against innoculation with live L2C cells. Separation by gel filtration, polyacrylamide gel electrophoresis, and isoelectric focusing indicate that the TSTA has an $M_r$ of ~12,500 and a pI of >10. It comigrates with cytochrome c in polyacrylamide gel electrophoresis but appears distinct from that molecule because removal of cytochrome c by affinity chromatography does not diminish TSTA activity and purified cytochrome c preparations have no TSTA activity. Trypsin treatment of purified preparations completely destroys TSTA activity. Current efforts now focus on the preparation of a monoclonal antibody to the TSTA using highly purified material as immunogen. An understanding of the chemical basis of this TSTA may be of great significance in understanding of the immunologic properties of B cell malignancies in humans as well as animals (Green and Gregg, LI, NIAID; Hearing, LCB/NCI; Maloy, LIG/NIAID).

Honors, Award and Scientific Recognition

Members of the Laboratory of Immunology play important roles in the national and international scientific community. They serve on editorial boards of many important journals. Dr. Paul is editor of the Annual Review of Immunology and is a member of the editorial boards of Immunological Reviews and The Journal of Molecular and Cellular Immunology. He is a member of the advisory board of the Journal of Clinical Immunology, is an advisory editor of the Journal of Experimental Medicine and an associate editor of Cell as well as being advisory editor for immunological diseases of the Cecil Textbook of Medicine. Dr. Shevach is a section editor for clinical immunology and immunopathology of the Journal of Immunology, a member of the editorial boards of Cellular Immunology, the Journal of Immunological Methods, and the Proceedings of the Society for Experimental Biology and Medicine and a member of the advisory editorial board of the Journal of Molecular and Cellular Immunology. Dr. Schwartz is a member of the board of reviewing editors of Science, of the advisory editorial boards of Immunology Today and the Journal of Molecular and Cellular Immunology, and of the editorial board of Stem Cells. Dr. Germain is a member of the advisory editorial board of the Journal of Molecular and Cellular Immunology. Dr. Green serves on the editorial board of Clinical Immunology and Immunopathology and on the editorial advisory board of Immunological Communications. Dr. Mage is a member of the editorial board of the Journal of Immunological Methods. Dr. Inman is an advisory editor of Molecular Immunology and is a member of the editorial board of Analytical Biochemistry.

Dr. Paul was elected Vice-President of the American Association of Immunologists and is a member of the Board of Directors of the Foundation for Advanced Education in the Sciences. He completed terms as a member of the Scientific Review Board of the Howard Hughes Medical Institute and as a member of the Scientific Advisory Committee of the New England Regional Primate Center. He is the chairman of the Board of Scientific Consultants of the Memorial Sloan-Kettering Cancer Center and of the Cambridge (UK) Branch of the Ludwig Institute for Cancer Research. Dr. Paul is a member of the Board of Scientific Advisors of the Jane Coffin Childs Memorial Fund for Medical Research, the Scientific Board of Visitors of the Oklahoma Medical Research Foundation, the Advisory Committee of the Howard C. Simmons Arthritis Research Center, the Scientific Advisory Council of the Cancer Research Institute, the Award Committee of the Lita Annenberg Hazen Awards for Excellence in Clinical Research, and the International Steering Committee of the Maimonides Conferences on Cancer Research.

During the past year, Dr. Paul was awarded the Distinguished Service Medal of the United States Public Health Service. He was the Culpeper Foundation Visiting Professor at the University of Michigan and presented the Robert A. Cooke Memorial Lecture to the American

Academy of Allergy and Immunology. He was an invited lecturer at the Maimonides Conference on B Lymphocytes at Ein Gedi, Israel, at the Spring Meeting of the British Society for Immunology, at the National Academy of Sciences Symposium on Biotechnology, and at the Boehringer-Ingelheim Centennial Symposium on Frontiers in Molecular Immunology. He chaired a minisymposium on Lymphokines at the annual meeting of the American Association of Immunologists.

Dr. Schwartz was the vice-chairman of the Gordon Conference on Immunochemistry and Immunobiology and was an invited speaker at the Neuroimmunology Symposium in Los Angeles, at the Symposium on Monoclonal Antibodies in Florence, Italy, at the Jane Coffin Childs Fund for Medical Research Symposium on Frontiers in Immunology, at the Johns Hopkins University Symposium on the T Cell Receptor, at the annual meeting of the Association for Clinical Histocompatibility Testing, at the Miami Winter Symposium, at the Cold Spring Harbor Symposium on Immune Recognition of Protein Antigens and at the Canadian Society for Immunology Symposium on Antigen Presentation.

Dr. Shevach is a member of the Research Committee and of the Research Fellowship Subcommittee of the Arthritis Foundation. He was an instructor in the Biology of Parasitism Course at the Marine Biology Laboratory in Woods Hole, MA, and chaired a session at the annual meeting of the American Society for Clinical Investigation and a workshop at the Leucocyte Culture Conference. He was a symposium speaker and session chairman at the Meeting on T Cell Differentiation held in West Germany, and was a symposium speaker at the annual meeting of the American Society for Microbiology.

Dr. Mage was an organizer of and an instructor in the FAES Advanced Immunology Course, and served as American Association of Immunologists representative to the board of the American Type Culture Collection. She presented the Simonetta Tosi Memorial Lecture to the Gruppe di Cooperazoine in Immunologia of the Italian Immunology Society, was the invited lecturer at the Fall, 1984 meeting of the D.C. Chapter of Sigma Xi, and is an invited participant in the Foundation for Microbiology Lecture Program.

Dr. Green was a lecturer at the European Immunological Societies' meeting in Israel and is a member of the NIAID Clinical Research Subpanel and of the NIAID Animal Care Committee.

Dr. Germain was a workship chairman at the Sixth Ir Gene meeting on "Transfection of MHC Genes" held in Oxford, UK, and was an invited speaker at The Fourth MHC Cloning Meeting at Stanford University and at the Gordon Conference on Immunochemistry and Immunobiology.

Dr. Margulies chaired a minisymposium on the Molecular Biology and Chemistry of MHC and Tla-Encoded Molecule at the American Association of Immunologists Annual Meeting and was an NIAID-NIH Visiting Professor at the University of Puerto Rico.

Dr. Inman was an invited lecturer at the Sixth International Symposium on Bioaffinity Chromatography and Related Techniques in Prague, Czechoslovakia, and at the Symposium on "Binding Reactions; Theory and Experiment" at Puebla, Mexico.

Dr. Mark Davis, a former post-doctoral fellow in the Laboratory of Immunology, received the Passano Award for his pioneering work on T cell receptor genes. Much of this work was performed in the Laboratory of Immunology.

## Administrative, Organizational and Other Changes

Miss Rose Lieberman, a senior member of the Laboratory of Immunology since 1968, retired this year after a career notable for a series of exceptional contributions to the fields of immunogenetics, immunochemistry and cellular immunology. Her colleagues organized a symposium in her honor in September of 1984 at which the principal speakers were Dr. Baruj Benacerraf, Harvard Medical School; Dr. Michael Potter, National Cancer Institute; Dr. Martin Weigert, Institute for Cancer Research, Fox Chase; Dr. Sheldon Dray, University of Illinois; and Mr. William Humphrey, Laboratory of Immunology, NIAID. This was followed by an evening reception in her honor. Miss Lieberman's retirement represents a very significant loss for the NIH scientific community. The members of the Laboratory of Immunology and her other colleagues at NIH express their best wishes to Miss Lieberman for her retirement.

Dr. Michail Sitkovsky has been appointed as a Visiting Scientist in the Laboratory of Immunology in a tenure-track position. He has established an independent research program on membrane events in lymphocyte activation and effector function.

The Laboratory of Immunology continues to play an important role in the training of young scientists. During the past year, an outstanding group of individuals completed their postdoctoral training in the Laboratory. They included John Ansel, Jonathan Ashwell, Drew Bentley, Samuel Breit, David Cohen, Nancy Francis, Linda Hillstrom, Zdenko Kovac, Lennart Logdberg, Thomas Malek, Hiroshi Narimatsu, Gustavo Ortega, Evelyn Rabin, Lawrence Samelson, Gen Suzuki, and Hiroshi Suzuki. Each of these scientists made substantial contributions to the research program of the Laboratory of Immunology.

Several postdoctoral fellows joined the Laboratory of Immunology for research training last year. They included: Mark Avigan, Letitia Carlson, Evan Gregg, Xu Han, Peter Hornbeck, Marc Jenkins, Kathryn Kimmel, Masanori Komatsu, Kathleen McCoy, Kazumasa Ogasawara, Drew Pardoll, Geraldo Pereira, Christian Peschel, Takashi Saito, Andrea Sant, Clifford Snapper, Hajime Takayama, and Wayne Yokoyama. Dr. Georg Stingl, Professor in the First Department of Dermatology at the University of Vienna Medical School, joined the Laboratory of Immunology for a sabbatical year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00030-17 LI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: W. E. Paul Chief LI, NIAID
Others: M. Brown, Guest Researcher, LI, NIAID; P. Hornbeck, Guest Researcher, LI, NIAID; J. Mizuguchi, Visiting Associate, LI, NIAID; J. Ohara, Visiting Associate, LI, NIAID; E. Rabin, LI, NIAID; W. Tsang, Medical Staff Fellow, LI, NIAID; R. Germain, Senior Investigator, LI, NIAID; D. Margulies, Investigator, LI, NIAID.

COOPERATING UNITS *(if any)* LIR, NIAID (J. Coligan and L. Maloy); IRLC, NHLBI (M. Beavan); USUHS (J. Mond and F. Finkelman); Columbia University College of P & S (S. Morrison); University of Texas, Southwestern Medical Center (E. Vitetta).

LAB/BRANCH
Laboratory of Immunology

SECTION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.25 | 4.5 | 1.75 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at understanding the mechanisms through which resting B lymphocytes are activated, stimulated to divide, and caused to differentiate into antibody secreting cells. During the past year, major emphasis has been placed on mechanisms through which membrane immunoglobulin transduces growth promoting signals and on the nature and mode of action of B cell stimulatory factor (BSF)-1. Evidence has been obtained that normal resting B cells display rapid increases in intracellular free $[Ca^{++}]$ and in inositol phosphates as a result of stimulation with anti-IgM and that inhibition of these processes is associated with inhibition of B cell responses strongly suggesting that increases in $[Ca^{++}]_i$ and protein phosphorylation through C kinase are critical steps in anti-IgM mediated B cell responses. Furthermore, BSF-1 has now been purified to a degree allowing an N-terminal amino acid sequence to be obtained and a monoclonal antibody to BSF-1 has been developed.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00035-10 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. K. Inman Senior Investigator LI, NIAID

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


The principal aim of this project is to test the hypothesis of general multispecificity for the combining regions of antibodies and other kinds of receptors. Receptor sites, according to theory developed earlier in this project, should be capable of interacting with virtually any substance in a manner that will lower the standard free energy of the system and thus exhibit an equilibrium association constant greater than 1. Most associations will be weaker than ones commonly measured, but occasional substances may bind to a receptor with affinities high enough to affect biological function. Their structures may not necessarily resemble those of the recognized effector, substrate or antigen.

The above hypothesis is being tested in the following way: Radiolabeled, monoclonal antibodies or solubilized receptors are passed through small, affinity chromatography columns. Accurate measurements are made of the retention (retardation) caused by a matrix-bound reference ligand in the presence and absence of many, diverse, suitably large compounds. The resulting retention values are employed directly in calculating association constants for these compounds and the receptor site. The distribution of constants provides a description of the receptor's multispecific character. The technique of quantitative affinity chromatography, developed in this study, provides a general and effective means for estimating very low to moderately strong association constants for antibodies and requires very small samples.

Knowledge of multispecific interactions will be employed in (1) re-evaluating general concepts of specificity (selectivity) in biological recognition and control, and (2) extending the scope of specific, affinity-based separations of receptor-bearing proteins. Special attention will be given to applying these findings to models of immune systems and control networks.




PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00036-20 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ig Genetics: Ontogeny & Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. G. Mage | Senior Investigator | LI, NIAID |
| Other: | E. Lamoyi | Visiting Associate | LI, NIAID |
| | N. McCartney-Francis | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

Basel Institute of Immunology, Basel, Switzerland (A. S. Kelus); LMB, NIADDK (G. A. Cohen, E. Padlan & D. Davies.); and LIB, NCI (T. Borsos & A. Circolo).

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.7 | 0.7 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although few, if any, Ig molecules bearing light chains of the K2 isotype are produced by our laboratory rabbits, we demonstrated transcription of the K2 gene by S1 protection analyses and light-chain sized mRNA by northern analysis with a K2-specific synthetic oligonucleotide probe. Similarly, although mutant Basilea rabbits produce little or no Igs with light chains of the K1-b9 type, we have detected low levels of b9 RNA. We are investigating whether this is aberrantly spliced message since we have found that the K1-b9 gene in Basilea rabbits has a point mutation in the splice acceptor site of the J-C intron. With similar methods, we have not however, detected mRNA or genomic DNA sequences corresponding to the b5 allotype that has been observed serologically to be produced by b9/b9 cells cultured in vitro with b5 anti-b9 and LPS. Thus alternative explanations for serological observations of latent allotypes must be invoked. We have also shown that synthetic oligonucleotides from the first and third framework regions (FR1 and FR3) of variable regions of heavy chains specifically distinguish mRNAs produced by a1 and a2 rabbits. The prototype FR1 and FR3 sequences may not, however, completely correlate with serologically detectable $V_H$a determinants. A strain of rabbits carrying the parental chromosome from which the Basilea mutant was derived has been developed and shown to have a restriction fragment length polymorphism (RFLP) of the K2 gene also found in Basilea rabbits. This allows linkage studies of the K1 phenotype, K2 RFLP and a newly discovered RFLP of the rabbit T cell receptor chain constant region gene. We have analyzed and predicted the locations of kappa light chain allotypic determinants. Predicted determinants were external, located in or near loops, and fell in two clusters of potentially interacting regions within which several overlapping sets of epitopes could occur. Interaction of anti-K1 antibodies with such epitopes on IgG anti-hapten antibodies abolished hapten-mediated dissociation of the antibody from hapten-coupled cells.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00147-10 LI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Mechanism of Activation of Thymus-Derived Lymphocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | R. H. Schwartz | Senior Investigator | LI, NIAID |
| Other: | L. Samelson | Research Expert | LI, NIAID |
| | H. Narimatsu | Visiting Associate | LI, NIAID |
| | H. Quill | Staff Fellow | LI, NIAID |
| | K. Ogasawara | Visiting Fellow | LI, NIAID |

COOPERATING UNITS *(if any)*

LBM, NIADDKD (J. Harford and R. Klausner)

LAB/BRANCH
Laboratory of Immunology

SECTION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.2 | 3.2 | 1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


An investigation of early biochemical events associated with T cell stimulation revealed that antigen- or concanavalin A-induced T cell activation results in the rapid phosphorylation of a 20kd protein that appears to be associated with the T cell antigen-specific receptor.




PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00148-10 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies of Guinea Pig $L_2C$ Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | I. Green | Senior Investigator | LI, NIAID |
| | Evan Gregg | Visiting Fellow | LI, NIAID |

COOPERATING UNITS (if any) LCB, NCI (V. Hearing); LIG, NIAID (L. Maloy); Medicine Branch, NCI (D. Longo & S. Bridges); Tenovus Research Laboratory, Southampton General Hospital, Southampton, England (F. Stevenson).

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 1.7 | .8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The $L_2C$ leukemia is a B cell leukemia of inbred strain 2 guinea pigs. These cells have surface IgM and C3 receptors. Studies have shown that these leukemia cells possess a strong tumor specific transplantation antigen (TSTA) that can easily be demonstrated by immunization protection tests in syngeneic animals. A procedure employing KCl extraction of the leukemic cell yields a soluble extract that is also highly antigenic. The physical and chemical properties of this soluble TSTA are now the subject of study. The findings to date indicate that this TSTA has several unusual properties; it has a M.W. of 12,500 (as determined by Sephadex chromatography and polyacrylamide gel electrophoresis); it is resistant to boiling for 5 minutes as well as to extremes of pH. Iso-electric focusing indicates that the immunogenic material has an iso-electric point >10. Treatment with trypsin, neuraminidase and periodate destroys the activity. The fraction containing the TSTA was pink suggesting that it might be cytochrome c. However, immunization with mouse cytochrome c (having the same sequence as guinea pig cytochrome c) failed to protect. Furthermore, immunization with cell fractionation of cells demonstrated that fractions containing membranes protected while the cytosol soluble fraction failed to protect. After removal of cytochrome c using a solid phase monoclonal antibody to cytochrome c, the material still contained the TSTA. Studies are continuing to fully characterize the biochemical nature of TSTA.




PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00223-04 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Interactions in the Immune Response

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | R. H. Schwartz | Senior Investigator | LI, NIAID |
| Others: | J. Ashwell | Medical Staff Fellow | LI, NIAID |
| | Z. Kovac | Visiting Fellow | LI, NIAID |
| | B. Fox | Guest Worker | LI, NIAID |
| | L. Carlson | Guest Worker | LI, NIAID |

COOPERATING UNITS *(if any)*

None

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Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.8 | 1.8 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Increasing the number of antigen-specific T cell clones in a T cell proliferation assay resulted in a shift in the antigen dose-response curves toward higher amounts of antigen (i.e. more antigen was required to achieve a given degree of stimulation). The antigen dose-response curve shifts were found to reflect the competition that occurred between the antigen-specific T cell receptors for their ligand, a combination of antigen and Ia molecule. This observation made it possible to determine if the difference in the potency with which several synthetic cytochrome c analogs could stimulate one cytochrome c-specific T cell clone was due to a difference in the avidity of the antigen-specific receptors on the T cell clone for the different Ia molecule-antigen combinations.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00224-04 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibodies as Probes for T Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. M. Shevach Senior Investigator LI, NIAID

Others: T. Malek, Research Expert; K. Gunter, Medical Staff Fellow; M. Honda, Visiting Associate; L. Lodgberg, Visiting Fellow; G. Perreira, Visiting Fellow; R. Kroczek, Guest Worker; K. Kimmel, Guest Worker

COOPERATING UNITS (if any)

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Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7.5 | 5.0 | 2.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

The major objective of our studies is to characterize cell surface structures on T and B lymphocytes as well as on non-T accessory cells (AC) which, in addition to the specific antigen receptor, are involved in the process of lymphocyte activation. Over the past 4 years we have developed a number of monoclonal antibodies (Mabs) to mouse T lymphocyte cell surface antigens which are capable of stimulating or inhibiting T cell triggering. One group of these reagents is directed to the receptor for interleukin-2 (IL-2) on activated murine lymphocytes. These Mabs have been used to further characterize the role of non-T AC in the induction of IL-2 receptor expression and to isolate and sequence a cDNA that contains the entire coding region of the murine IL-2 receptor. A new lymphokine, IL-2 inhibitor, which is capable of neutralizing the biologic activity of IL-2 has been characterized and purified to apparent homogeneity. Newly developed Mabs to the Thy-1 antigen were used to demonstrate the critical role of this molecule in signal transduction following transfection of the Thy-1 gene into human cells. Lastly, we have shown that the L3T4 antigen, a marker for a subpopulation of T cells, may play a dual role in T cell function by interacting with target molecules on AC and by subsequently functioning as a signal transmitter to other cell surface molecules on the T cell. The ultimate goals of our studies are to fully understand the regulatory mechanisms that control T cell activation and differentiation. Mabs to lymphocyte surface antigens should prove to be useful tools in these studies and may also prove to be attractive candidates for in vivo therapeutic use in attempts to modulate or abrogate an ongoing immune response.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00225-03 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of UV Light on the Course of Auto-Immune Dis. in Auto-Immune Strains in Mice.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | I. Green | Senior Investigator | LI, NIAID |
| Others: | J. Ansel | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

Arthritis Branch, NIADDKD (A. Steinberg and J. Mountz)

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00226-04 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below tha Principal Investigator.) (Name, title, leboretory, and institute effiliation)

| | | | |
|---|---|---|---|
| PI: | R. G. Mage | Senior Investigator | LI, NIAID |
| Others: | E. Lamoyi | Visiting Associate | LI, NIAID |
| | M. McCartney-Francis | Guest Worker | LI, NIAID |
| | M. Komatsu | Guest Worker | LI, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.3 | 1.7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


We sought a molecular explanation for the loss of expression of the normally predominant K1-b9 type of kappa light chains in Basilea rabbits. No major deletions or rearrangements associated with Cκ1 were found by Southern analyses. We cloned and sequenced the Cκ1 gene and its 3' and 5' flanking regions and found a G to A transition in the acceptor splice site of the Jκ-Cκ intron. This change in an invariant AG/ to AA/ provides a molecular explanation for the loss of K1 light chain expression.

We cloned and sequenced VH genes from a rabbit of a3 phenotype in an attempt to understand the molecular basis for serological observations of latent VHa allotype expression. We found rabbit VH genes that were unusually close (~3kb apart) and encoded unusual combinations of allotype-correlated codons. It now appears unlikely that all rabbits have the same genomic content of structural genes for VH regions or that the allelic behavior of VH allotypes simply reflects regulatory genes. Since most alternative amino acids at allotype-correlated positions can be derived from each other by single-base changes, somatic mutations and/or gene-conversion-like events may explain at least some observations of latent VHa allotypes. The proximity of rabbit VH genes may enhance gene-conversion-like events.

We cloned and sequenced two thymus-derived cDNA clones that encode the constant region of the rabbit T cell receptor β chain and found high homology to the human and murine sequences. One cDNA encodes an unusual non-Vβ sequence 5' of Cβ that would produce a protein with unknown function. The other encodes a rabbit Vβ with 72.5% homology to a mouse Vβ. In addition, the sequence of a corresponding single-copy genomic Vβ from a second rabbit had an identical sequence suggesting that no somatic mutations have occurred in the expressed Vβ gene.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00229-04 LI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Lymphocyte Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | W. E. Paul | Chief | LI, NIAID |
| Others: | D. Cohen | Medical Staff Fellow | LI, NIAID |
| | E. Nielsen | Biologist | LI, NIAID |

COOPERATING UNITS (if any) Dept Med. Microbiology, Stanford University School of Medicine, Stanford, CA (M. Davis); Dept Biology, University of California-San Diego (S. Hedrick); A+R, NIADDKD (J. Siegel and A. Steinberg), and MET, NCI (T. Waldmann).

LAB/BRANCH
Laboratory of Immunology

SECTION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2 | 1 | 1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to study genes regulating the growth and differentiation of lymphocytes. Our approach has been to isolate genes which are specifically expressed in B or T lymphocytes through the technique of substracive hybridizaion (see Methods). We have concentrated on isolating genes encoding cell surface receptors or genes which can be studied in the context of mutations affecting lymphocyte development. We have been successful in isolating cDNA clones encoding the α and β chains of the T cell's receptor for antigen. We have also defined an x-linked family of mouse genes which may be mutant in mice bearing the X-linked immunodeficiency, xid, and we have begun to define human equivalents to this gene.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00259-04 LI |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Ia Molecules and Immune Response Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)

| | | | |
|---|---|---|---|
| PI: | W. E. Paul | Chief | LI, NIAID |
| Others: | M. Brown | Guest Researcher | LI, NIAID |
| | R. N. Germain | Senior Investigator | LI, NIAID |

COOPERATING UNITS (if any)
Harvard School of Public Health, Boston, MA. (L. Glimcher)

LAB/BRANCH
Laboratory of Immunology

SECTION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .75 | .25 | .50 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class II major histocompatibility complex (MHC) gene products are membrane glycoproteins, expressed mainly on macrophages and B cells, which are co-recognized with antigen by helper T cells and related L3T4$^+$ T cells. T cells distinguish the polymorphic forms of class II molecules in such a way that, in most cases, they corecognize only one allelic form of the product of a given class II locus. The structural basis of this restriction in coregnition function may depend on amino acids in the class II molecule which act as contact residues for antigen (desetopes) or for the T cell receptor (histotopes). In order to analyze this, structural mutants of I-A$^k$-bearing B cell-B lymphoma hybridomas have been selected. These mutants have altered capacity to present antigen to T cell hybridomas. $A_\beta$ genes have been cloned from a pair of sibling mutant lines and shown by DNA-mediated gene transfer to be responsible for the altered antigen-presenting phenotype. These mutant genes have a single nucleotide change leading the replacement of glutamic acid with lysine at position 67 in the β chain of the I-A$^k$ molecule. These results demonstrate that even a single residue change can have profound effects on antigen-presenting phenotype and demonstrate that the hypervariable region around position 67 of the β chain is very important in the function of I-A class II molecules.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00349-03 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. N. Germain Senior Investigator LI, NIAID

Others: M. A. Norcross, Medical Staff Fellow, LI, NIAID; N. S. Braunstein, Medical Staff Fellow, LI, NIAID; J. Miller, Guest Worker, LI, NIAID: F. Ronchese, Guest Worker, LI, NIAID; R. Lechler, Guest Worker, LI, NIAID; M. A. Brown, Guest Worker, LI, NIAID; D. H. Margulies, Investigator, LI/NIAID; W. E. Paul, Chief, LI/NIAID.

COOPERATING UNITS *(if any)*

Harvard School of Public Health, Boston, MA (L. H. Glimcher); IB, NCI (A. Singer).

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.6 | 3.0 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Class II (Ia) gene products play critical roles in a variety of T lymphocyte responses. They are the primary stimulating antigens in allogeneic and syngeneic mixed lymphocyte responses, they "restrict" recognition of foreign antigens by L3T4$^+$ T lymphocytes, and they control the ability of animals to respond to T dependent antigens (immune response [Ir] gene function). A combination of immunological and molecular genetic approaches is being used to gain an understanding of the structural basis for this recognition of Ia by T lymphocytes. Towards this goal, genomic or cDNA clones of various allelic forms of $A_\beta$, $E_\beta$, $A_\alpha$, and $E_\alpha$ have been isolated, and where necessary, sequenced. $A_\alpha$ and $A_\beta$ genes have been transfected into B lymphomas or L-cells, and Ia expressing transformants obtained. These have been used to stimulate a variety of T cell hybridomas and clones, establishing the importance of both $A_\alpha$ and $A_\beta$ polymorphic regions in forming restriction elements. Exon-shuffling between allelic $A_\beta$ genes localized both serologic and T cell recognition sites to the highly variable $\beta_1$ domain. Sequence analysis of EMS induced $A_\beta$ mutants also localized a critical site of function to a small region of the $\beta_1$ domain near the recently sequenced bm12 mutation. Finally, attempts to construct transfectants expressing "hybrid" I-A molecules revealed an unexpected restriction on $\alpha$:$\beta$ chain assembly which maps to the $\beta_1$ domain. These results have shown the validity of this approach in determining the critical structural features of class II molecules recognized by T lymphocytes, and provided new insights into the molecular basis of Ia chain assembly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00394-02 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of Lymphocyte Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. H. Margulies | Senior Investigator | LI, NIAID |
| Others: | M. McCluskey | Visiting Scientist | LI, NIAID |
| | L. Hillstrom | Guest Worker | LI, NIAID |
| | L. Boyd | Chemist | LI, NIAID |
| | R. Germain | Senior Investigator | LI, NIAID |
| | D. Cohen | Medical Staff Fellow | LI, NIAID |

COOPERATING UNITS *(if any)* LIG, NIAID (W. L. Maloy and J. Coligan); Immunology Branch, NCI (H. Golding, A. Singer and J. Bluestone); Dept Biochemistry, NYU Medical School (P. d'Eustachio)

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: 2.5 | PROFESSIONAL: 1.5 | OTHER: 1.0 |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of our continuing effort to understand the structure-function relationships of cell surface molecules involved in the immune response, we have been involved in a number of studies applying the methods of recombinant DNA technology to genes that encode the major histocompatibility antigens of the mouse as well as to those that encode molecules linked to the Mls locus on chromosome 1. In particular, we have focussed on: A. Generating in vitro recombinant chimeric class II/class I genes, and analyzing their expression biochemically, structurally, and functionally; B. Generating in vitro deletion mutants of the class I MHC genes H-2L$^{d}$ and H-2D$^{d}$ and analyzing their expression biochemically, structurally, and functionally; C. Analyzing the protein products derived from alternatively spliced mRNAs of H-2L$^{d}$ and H-2D$^{d}$ deletion mutants; and D. Developing a molecular biological approach to the cloning of genes linked to and/or encoding the Mls locus of the mouse, the only known non-major histocompatibility complex locus controlling a primary T cell proliferative response.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00400-02 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunoregulatory Disorders in Systemic Lupus Erythemotosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PL: | I. Green | Senior Investigator | LI, NIAID |
| Others: | H. Suzuki | Visiting Fellow | LI, NIAID |
| | K. Nakanishi | Visiting Fellow | LI, NIAID |

COOPERATING UNITS *(if any)*

Arthritis Branch, NIADDK (A. Steinberg)

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 0.9 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The proliferative response of B lymphocytes to stimulation with anti-IgM antibodies and B cell growth factors was studied in 27 patients with SLE and 17 normal donors. In addition, the expression of messenger RNA of the proto-oncogene c-myc was also studied in B cells from SLE patients and normal donors. The proliferative response of lupus B cells to anti-IgM and B cell growth factors as compared to normal B cells, demonstrated a wide range of response. Ten were lower than normal and 8 were either normal or supernormal. As compared to normals, expression of B cell c-myc RNA from SLE patients was either normal or depressed. In general, in patients with SLE there was a positive correlation between levels of c-myc expression and degree of proliferation in B cells after stimulation with anti-IgM and B cell growth factors.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00403-02 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetic Analysis of T Cell Receptor Structure and Repertoire

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: R. N. Germain | Senior Investigator | LI, NIAID |
| Others: J. Miller | Guest Worker | LI, NIAID |
| R. Lechler | Guest Worker | LI, NIAID |
| E. Shevach | Senior Investigator | LI, NIAID |
| R. Schwartz | Senior Investigator | LI, NIAID |

COOPERATING UNITS *(if any)* LMI, NIAID (B. Folkes); MET, NCI (W. Greene); Harvard Medical School (M. Dorf).

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 0.5 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three distinct types of cell surface molecules play major roles in T lymphocyte activation and growth. Clonally distributed receptors provide the structural basis for selective antigen-specific regulatory and effector activity of T cells. Other non-clonally distributed molecules, present on resting T cells, appear to play a role in the triggering process. The Thy-1 molecule is one such structure. Finally, molecules appearing only on activated T cells participate in differentiation and clonal expansion, chief among these being the interleukin-2 (IL-2) receptor. To investigate the structure, function and regulated expression of these three classes of molecules, recombinant DNA clones of each have been isolated, sequenced, and used in DNA-mediated gene transfer experiments, or as hybridization probes for DNA and RNA blotting analysis of various cell types. This work has shown 1) a sequential appearance of the T cell receptor $\beta$, then $\alpha$ chains during T cell ontogeny in the thymus, 2) an unusual pattern of T cell receptor gene rearrangement and expression in T suppressor cells, 3) the ability of mouse Thy-1 to act as a mitogenic signal site in human T cells, 4) the structure of the mouse IL-2 receptor, and 5) indicated a complex relationship between this structure and IL-2 receptor function.



PHS 6040 (Rev 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 000426-01 LI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Organization of the Antigens in the Plasma Membranes of the Lymphocyte Clones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michail V. Sitkovsky | Visiting Scientist | LI, NIAID |
| Other: | H. Takayama | Visiting Fellow | LI, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunology

SECTION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | .75 | .5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conjugate formation between cytotoxic T lymphocytes (CTL) and target cells (TC) results in dramatic consequences for both. TC will be destroyed and CTLs receive a biochemical signal for activation and start to proliferate and to release lymphokines. Several proteins on the surface of CTL were implicated in such interactions (e.g. LFA-1, T200, Lyt2.2, T cell receptor-T3 complex), but it is not known how they are organized on the surface of CTL, although we have circumstantial evidences that T cell receptor-T3 molecules are part of a bigger multimolecular complex. In the studies started at the NIH, we are trying to evaluate distribution of different molecules in the lymphocyte plasma membrane and to demonstrate multimolecular complexes on the surface of CTL using whole cloned cells, purified preparations of plasma membranes, monoclonal antibodies, and heterobifunctional crosslinking reagents. Preliminary results suggest that there is an asymmetrical distribution of surface proteins between different domains of lymphocyte plasma membranes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00427-01 LI

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Mechanisms of the Antigen-Specific and Antigen-Nonspecific Cellular Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Michail V. Sitkovsky | Visiting Scientist | LI, NIAID |
| Others: | H. Takayama | Visiting Fellow | LI, NIAID |

COOPERATING UNITS *(if any)*
LCM, NHLBI (R. Kincaid)

LAB/BRANCH
Laboratory of Immunology

SECTION
National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

INSTITUTE AND LOCATION

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | .75 | I |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The major steps of the process of interaction between cytotoxic T lymphocytes (CTL) and target cells (TC) are $Ca^{++}$-dependent. Presence of $Ca^{++}$ is required for CTL-TC conjugate formation and increase in the intracellular free $Ca^{++}$ concentration is documented as an early feature of T-cell activation through T cell-receptor-T3 complex on the cell surface. It is not yet known, however, which proteins on the lymphocyte surface have $Ca^{++}$-binding, $Ca^{++}$-channel forming properties and $Ca^{++}$-dependent functions. It is also not known which biochemical pathways are involved in T cell activation following an increase in concentration of intracellular $Ca^{++}$. This project deals with these questions. We are attempting to isolate and to identify $Ca^{++}$-binding and $Ca^{++}$-dependent, calmodulin-regulated proteins in the cytoplasm and different domains of the plasma membranes of lymphocytes exploiting known physico-chemical properties of $Ca^{++}$-binding proteins. Preliminary results indicate that mouse spleen lymphocytes appear to contain low levels of a calmodulin-dependent phosphatase, which is immunologically crossreactive and has similar molecular weight with a $Ca^{++}$-dependent phosphatase from bovine brain, previously known as calcinueurin.




PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF IMMUNOREGULATION
1985 Annual Report
Table of Contents

Summary Report
Laboratory of Immunoregulation
October 1, 1984 through September 30, 1985

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID

## Studies of the Human B Cell Cycle: Identification, Isolation, Purification, and Characterization of Human B Cell Growth and Differentiation Factors

Over previous years, we have developed a model system for the dissection of the events involved in the driving of human B cells from the resting state through proliferation and ultimately to terminal differentiation and antibody secretion. We delineated the precise steps in the human B cell cycle and described the role of B cell growth factors (BCGF) and B cell differentiation factors (BCDF) in the various stages of the B cell cycle. Over the past year (1984-1985), we have identified and isolated a high molecular weight (HMW) BCGF (60 kd) which was produced by a human B cell lymphoma line as well as by a T cell acute lymphoblastic leukemia (ALL) line. The factor preferentially enhanced the proliferation of preactivated normal human B cells and hence provided a signal later on in the sequential steps of the B cell cycle; the factor also enhanced the proliferation of certain B cell lines. The HMW-BCGF can be distinguished clearly from other lymphokines known to enhance B cell proliferation such as interleukin (IL)-1, IL-2, and interferon (IFN). We purified the HMW-BCGF to homogeneity and prepared large amounts of highly purified material for amino acid sequencing. In addition, we have prepared cDNA libraries from HMW-BCGF-producing cell lines for use in attempts at cloning the gene for BCGF. The purified material is also being used in studies aimed at identifying the cellular receptor for BCGF.

A monoclonal antibody directed against the HMW-BCGF was developed which specifically inhibited the activity of HMW-BCGF in enhancing B cell proliferation, specifically bound to HMW-BCGF in Western blots, specifically absorbed HMW-BCGF activity from culture supernatants, and specifically absorbed an internally labeled protein from T-ALL supernatants which comigrates with HMW-BCGF on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The antibody will be employed in cloning the gene for HMW-BCGF and in further exploring the physiologic role of HMW-BCGF.

In addition to the malignant human B cell line mentioned above whicn produced a HMW-BCGF, we also demonstrated that normal peripheral blood B cells produced a BCGF which might serve an autocrine function. This observation has important potential implications in understanding the T cell-independent expansion of the human B cell repertoire. In this regard, we studied a unique malignant human B cell line which was composed of cells which either produced or responded to BCGF as determined by cloning. Both producer and responder clones had identical gene rearrangements by Southern blot analysis, so they must have been derived form the same progenitor cells. It is unclear why certain clones acquire the ability to constitutively produce BCGF. However, this phenomenon may explain how certain B cell malignancies develop as well as certain autoimmune diseases characterized by hyperactive B cells.

In 1983 we demonstrated that normal human B cells can be induced to express receptors for IL-2 as determined by binding of anti-Tac antibody. In addition, we demonstrated that activated B cells proliferated in response to recombinant IL-2. Over the past year, we demonstrated that IL-2 could enhance proliferation of activated B cells as well as serve as a differentiation factor for activated B cells. When activated B cells were separated into Tac+ and Tac- populations, the Tac+ cells responded to IL-2, BCGF, and BCDF, while Tac- cells were only responsive to the classic B cell-specific factors BCGF and BCDF. In addition, we demonstrated that γ-IFN synergized with IL-2 in the induction of differentiation of activated B cells, although it could not do so alone. Finally, we confirmed the induction of IL-2 receptors on B cells by various activation signals by demonstrating the transcription of mRNA for IL-2 receptors in B cells in response to activation.

We demonstrated that different types of BCGF, i.e., HMW-BCGF and low molecular weight (LMW)-BCGF, induced differential proliferation in various subsets of B cells. When B cells were cultured in Marbrook vessels for 3 months, the surviving cells responded preferentially to HMW-BCGF and not to LMW-BCGF. In addition, B cells from patients with common variable hypogammaglobulinemia responded well to LMW-BCGF, but very poorly to HMW-BCGF.

Finally, we have undertaken studies aimed at delineating the BCGF receptor. We have covalently coupled iodinated highly purified BCGF to the surface of activated B cells to examine the binding site of BCGF. Also, we have developed monoclonal antibodies to activated B cells which functionally block the interaction of BCGF within the cell, but do not bind to cell surface antigens on T cells or monocytes. (Ambrus, Kehrl, Volkman, T. Nakagawa, N. Nakagawa, Lê thi Bich-Thuy, Tomita, Muraguchi, Jurgensen, Mostowski, Fauci, LIR/NIAID; Greene, MB/NCI.

<u>Studies on Clones and Transformed Human Lymphocytes: Model System for the Dissection of Lymphocyte Subset Function</u>

A number of phenotypically defined cloned subpopulations of human T cells were developed, and it was demonstrated that these clones manifested restricted functional helper activity which was related to the secretion of selected B cell tropic factors. For example, the clone YA2 is a T4+/Leu 8+ potent direct helper only to B cells that are preactivated and proliferating due to its selective secretion of a differentiation factor and not an activation or growth factor. These panels of clones should prove useful in the dissection of the role of individual T cell subsets in the regulation of the human B cell cycle.

Antigen-specific T cell clones were directly infected and transformed with human T lymphotropic virus (HTLV)-I. Following transformation, these T cells proliferated spontaneously without exogenous antigenic triggering and produced γ-IFN, BCGF, and BCDF constitutively. IL-2 was not secreted by the transformed cell line, whereas the uninfected line made significant levels of this lymphokine. In addition, viral infection markedly upregulated the expression of IL-2 receptors, which allowed the cell to effectively absorb exogenous IL-2 from cultures. The ability of viral infection to selectively abrogate the synthesis of one lymphokine while the cell is constitutively proliferating and producing at least three other factors implies separate regulatory mechanisms for these functions. In addition, the capacity of this

virus to cause a transformed T cell to synthesize and express heightened levels of IL-2 receptors and absorb available IL-2 efficiently may be fundamental to the capacity of the virus to cause lymphoproliferative disease in vivo and to blunt the ability of normal T cells to respond appropriately to antigenic stimuli. Finally, the dissociation of proliferation from stimulatory signals appears to be essential for similar transformed cells to escape immunoregulatory influences in vivo.

HTLV-I was also employed to infect B cells and to develop B cell lines which were not Epstein-Barr virus (EBV) transformed. These lines were subsequently cloned and were shown to express IL-2 receptors and differentiate in response to IL-2. Such clones will be useful in delineating precisely the differentiation steps in the human B cell cycle. (Volkman, Goldstein, Tomita, Fauci, LIR/NIAID; Gallo, Popovic, LTCB/NCI)

Pharmacologic Modulation of the Human Immune Response

Over the last 13 years, the LIR has been engaged in the study of the effect of corticosteroids (CS) on the human immune response. Over this past year, we have examined the differential effects of in vitro CS on various stages in the B cell cycle. A gradation of effect was noted on the discrete phases of B cell activation, proliferation, and differentiation, whereby the earliest activation step in the cycle was most sensitive to the suppressive effects of CS. We further demonstrated that CS preferentially blocks the protein kinase-dependent signal transmission in $G_0$ phase B cells. These studies have provided new insights into the complex nature of CS-induced modulation of human B cell responses.

In 1983-1984 we reported that cyclosporin A (CsA) selectively suppressed an early step in human B cell activation and had relatively little inhibitory effect on the subsequent factor-dependent proliferation and differentiation. Over the past year, we demonstrated a differential effect of CsA on the secretion of IL-2 versus BCDF by T cells; similar concentrations of CsA resulted in maximal inhibition of IL-2 production, but did not suppress secretion of BCDF.

Transforming growth factor (TGF)-β is a polypeptide that in conjunction with epidermal growth factor has been shown to stimulate reversible transformation of non-neoplastic murine fibroblasts in soft agar. We demonstrated that TGF-β markedly suppressed IL-2-dependent T cell proliferation and BCGF-dependent B cell proliferation. In addition, it dramatically inhibited BCDF-dependent Ig production, IL-2-dependent upregulation of IL-2 and transferrin receptors on T cells, and natural killer (NK) cell activity of large granular lymphocytes. Thus, TGF-β may serve as a potentially important immunoregulatory protein in the human system.

Finally, two neuropeptides were demonstrated to have reproducible effects on the proliferation and differentiation of human B cells. Adrenocorticotropin hormone (ACTH) and β endorphin increased the proliferation of activated B cells when present in culture, with either a BCGF or recombinant IL-2, whereas neither had a significant effect in the absence of the growth factor. Studies are in progress to characterize the binding sites for these neuropeptides on human B cells. (Bowen, Kehrl, Alvarez-Mon, T.

Nakagawa, N. Nakagawa, Fauci, LIR/NIAID; Sporn, LCP/NCI; Cupps, Georgetown University)

Molecular Biologic Approach to Immunoregulation

The activation process of T lymphocytes was characterized from an intracellular, molecular standpoint. The c-myc oncogene, the IL-2 growth factor, and the IL-2 receptor genes are known to be induced during the early activation of T cells. We have determined regulatory regions within the chromatin structure of these genes which allow us to dissect the nuclear events at the molecular level which result from the transmission of the extracellular activation signal. Along the same lines, a model system has been developed to study the effect of differentiation at the nuclear level. The promyelocytic leukemia cell line HL60 can be differentiated terminally in vitro. Concomitantly, the c-myc oncogene is downregulated and its chromatin changes dramatically. We have also initiated more broadly based studies directed at the activation process of T cells. cDNA libraries were made from activated peripheral blood T cells in order to identify genes which are specifically and immediately induced upon mitogenic stimulation. A similar approach has been utilized in order to identify the genes coding for the lymphokines which are elaborated by these T cells after stimulation and in particular to identify the gene for the well-characterized BCGF. Gene cloning of this factor has been pursued also by preparing distinct cDNA libraries from a B cell tumor which secretes the factor constitutively. These libraries allow screening for the growth factor by two methods: one is based on partial amino acid sequence information of the purified growth factor and the other method is based on the detection of BCGF by polyclonal antibodies in an expression library. (Siebenlist, Bressler, Ambrus, Zipfel, Fauci, LIR/NIAID; Kelly, IB/NCI; Greene, Leonard, MB/NCI; Holbrook, LP/NCI; Crabtree, Stanford University)

Studies in the Acquired Immunodeficiency Syndrome (AIDS)

Over the past 3½ years, beginning soon after the recognition of the AIDS epidemic, the LIR has been intensively involved in the study of this disease from several perspectives. Over the last year, we have continued and greatly intensified our studies in the areas of epidemiology, clinical aspects, virology, immunology, specific therapy, and immunologic reconstitution.

From an epidemiologic standpoint, we have prospectively followed over 500 employees of the NIH Clinical Center (where we have seen more than 150 patients with AIDS) for the development of evidence of infections with the AIDS retrovirus. This was done utilizing the techniques of enzyme-linked immunosorbent assays and Western blotting to detect antibodies against HTLV-III/lymphadenopathy associated virus (LAV), immunologic monitoring, and virus isolation procedures. Our cohort of 500 health care workers included 40 individuals who had experiences with either needle stick injuries or mucosal splashes from AIDS patients. Except for three individuals, each of whom belonged to an established risk group, there was no evidence of serologic or immunologic alterations suggestive of infection with the AIDS retrovirus. These latter individuals were antibody positive and exhibited subtle abnormalities of immune function. These observations provided extremely important evidence that the transmissibility of this agent is clearly restricted, is not related to casual or even close contact typical of that

seen between patients and health care workers, and is limited primarily to sexual contact or via blood or blood products from an infected individual. We have extended our epidemiologic studies to the families of AIDS patients, particularly blood product-related cases, and to pregnant women in risk groups and their newborn infants.

A major observation was made this year concerning the persistence of virus in a latent form in human lymphocytes. We developed a human T cell line (A3.01) which is T4/Leu 3 positive and is easily infected with the AIDS virus in a manner similar to the infection of peripheral blood Leu 3+ cells. Ten days following inoculaton of this cell line with the virus, there was a peak in detectable reverse transcriptase activity concomitant with a loss of the Leu 3 marker, slowing of cell growth, and cell death. At approximately 14 to 21 days, a Leu 3- cell began to grow out of cultures which were not actively producing virus, but which could be induced to produce virus by exposure to 5-iodo-2'deoxyuridine, 5 azacytodine, or $\gamma$ irradiation. In the absence of these latter inductive signals, the cells grew essentially indefinitely in culture without secreting detectable virus. The emergence of this survivor cell line has important implications as a model for latent retroviral infection in man.

The A3.01 cell line has also proved to be an excellent system to study the biology and molecular biology of the AIDS retrovirus. By employing Western and Northern blotting techniques, we determined the precise sequence of mRNA expression and synthesis of viral proteins.

We have utilized our ability to mimic in vitro the retroviral infection with the A3.01 line to examine potential inhibitors of viral replication. In this manner, we have screened several biologics, including IFN and antibodies, for antiviral activity.

In collaboration with Dr. Malcolm Martin (LMM, NIAID), we have molecularly characterized by restriction enzyme analysis and DNA cloned several different geographically distributed isolates of the virus. Comparison of such isolates have revealed a gradation of heterogeneity in genome largely within the region of the viral envelope gene. In this regard, isolates from Central Africa were significantly different from a variety of North American isolates. Differences were seen also in biologic properties, especially in terms of growth rate and cytopathic effects.

Studies on the molecular structure of the virus have led to the development of an infectious clone which currently is being propagated in a rhabdomyosarcoma cell line. Studies utilizing this infectious clone will surely yield important information on the pathophysiology of the AIDS retrovirus. In addition, we have developed labeled DNA probes for the virus which we have employed in in situ hybridization studies to detect infection at the single cell level. Using these techniques, virus has been identified in peripheral blood, bone marrow, and semen, and we will use this approach to monitor our studies on antiviral therapy and immunologic reconstitution.

In collaboration with Dr. Hardy Chan (Syntex, Inc.), a variety of DNA clones have been expressed in prokaryotic systems. The proteins which are expressed should provide excellent reagents for the study of the precise nature of the immune response to the AIDS retrovirus.

A variety of monoclonal antibodies against individual viral components have been developed. At least six of these appear to be direct against components of the viral envelope glycoprotein. It is anticipated that these types of reagents may have potential clinical relevance in that they may block the activity of the virus. In addition, production of monoclonal antibodies of this sort should allow for the more precise characterization of the synthesis of viral proteins.

The LIR was the first laboratory to transmit the infection to a nonhuman primate. We inoculated chimps with plasma from patients with the lymphadenopathy syndrome and two chimps develped antibody to HTLV-III/LAV; one of the chimps also developed transient lymphadenopathy. This accomplishment has potentially important implications for the establishment of an animal model for the testing of therapeutic and vaccine candidates.

The area in which the LIR has placed its major effort in AIDS has been the study of the underlying immune defect. We were the first laboratory to precisely delineate the quantitative as well as the qualitative abnormalites of T4 cells. In addition, we were the first to demonstrate that B cells from AIDS patients were not normal as had been previously described. In fact, they exhibited polyclonal hyperactivity due to either stimulation with opportunistic viruses such as cytomegalovirus (CMV) and EBV, coinfection of the B cells with EBV and HTLV-III/LAV, a lack of normal suppressor mechanisms related to a deficiency of the T4 subset responsible for the induction of suppression, or a combination of these. Over the past year, we extended our observations on the selectivity of the T cell defect by demonstrating that the subset of T4 cells which is responsible for antigen recognition is defective early in the disease and preferentially. This defect results either from a selective quantitative depletion of this subset or a functional defect within this subset due perhaps to latent infection with the HTLV-III/LAV. Contributing to the defect in the response to specific antigen is the fact that monocytes from AIDS patients are defective in their ability to present soluble antigen to syngeneic T cells. We demonstrated this point by experiments involving measurement of antigen responsiveness of T cells from a normal donor cocultured with monocytes from his identical twin with AIDS. In addition, utilizing monoclonal antibodies which recognize nonoverlapping subsets of T4 cells, we generated phenotypic data consistent with the functional observations that the antigen-reactive subset is selectively deficient in AIDS patients.

We also demonstrated that from an immunologic standpoint there is a gradation of severity of immune defects which correlates with the clinical subgroups of patients. Patients with opportunistic infections have more severe immune defects than those with Kaposi's sarcoma, who in turn have more severe defects that those with the lymphadenopathy syndrome. This finding has important implications for the design and stratification of clinical trials, especially those which employ immunomodulators.

In addition to our demonstration that the T4 cell was selectively infected in vitro by the virus and also was the subset from which the virus could be selectively isolated in specimens from patients with AIDS and AIDS-related complex, we further demonstrated that purified human monocytes could be infected with the AIDS retrovirus suggesting that the monocyte could serve as an important reservoir for the virus, similar to the situation

encountered with the Visna virus of sheep which is genomically similar to the AIDS retrovirus.

We had originally demonstrated that patients with AIDS had a defect in CMV-specific cell-mediated cytoxicity as well as NK cell function, the latter being correctable in vitro with IL-2. This observation served as a rationale for our use of IL-2 in clinical trials. Over the past year, we have shown that this enhancement of NK activity was due to a γ-IFN-independent mechanism. This has important implications in determining whether or not multiple lymphokines may be required for adequate in vivo immunoenhancement. Additional studies of cytotoxic capabilities of lymphocytes and antibodies from HTLV-III-infected individuals against virus-infected cells demonstrated that blood lymphocytes from patients with early AIDS syndromes exhibited increased cytoxicity against these targets and that sera from antibody-positive asymptomatic patients mediated substantial antibody-dependent cellular cytoxicity against HTLV-III infected cell lines.

The LIR is also involved heavily in the treatment of AIDS patients with respect to the underlying retroviral infection, the opportunistic infections, and the underlying immune defect. Substantial advances have been made in each of these areas. CMV is an extremely important opportunistic pathogen in AIDS patients, causing severe necrotizing retinitis, progressive pneumonitis, and debilitating colitis. Up to this point, there has been no effective treatment for CMV infections. Over the past year, we have treated 18 patients with dihyroxymethylpropoxymethylguanine (DHPG) who had severe CMV infections. All three clinical manifestations of CMV were arrested in these patients, particularly retinitis. A major problem has been that remissions are short-lived and maintenance therapy is required. However, this is the most effective treatment known for CMV.

In collaboration with Dr. Samuel Broder (NCI), we have had considerable success in inducing resolution of HTLV-III viremia in AIDS patients by the administration of suramin, which is an inhibitor of reverse transcriptase, the enzyme used by the virus to transcribe viral RNA to a DNA copy required for its replication. Although four of four patients had resolution of their viremia, all had recrudescence following discontinuation of drug. Nonetheless, these studies are extremely important in our future plans to combine specific antiviral therapy with immune reconstitution. Other agents which will be tested are HPA-23 and foscarnet.

Finally, major attempts have been made in the LIR to reconstitute the immune system of AIDS patients. We have had some potentially important favorable results with the use of high doses of recombinant IL-2. We noted enhanced spontaneous lymphocyte proliferation, elevation in total lymphocyte count, and decline in polyclonal B cell activation in patients receiving IL-2. In addition, gross and histologic evidence of regression of Kaposi's sarcoma lesions was noted.

The LIR was the first laboratory to investigate the potential role of cellular reconstitution of the immune system in AIDS by studying bone marrow transplantation and lymphocyte transfusions in identical twins. We performed the first identical twin bone marrow transplant and demonstrated transient improvement in immune function. The partially reconstituted immune system declined similar to the original one, almost surely because of the persistence

of the lymphocytopathic retrovirus in the recipient. Based on this experience, we will combine suramin antiretroviral therapy with bone marrow reconstitution. We are currently treating three AIDS patients who have healthy identical twins with suramin in preparation for subsequent bone marrow transplantation. (Lane, Folks, Koenig, Rook, Lightfoote, Fauci, LIR/NIAID; Masur, Alter, Henderson, CC; Broder, Gelmann, CDP/NCI; Martin, Rabson, Benn, Gendleman, LMM/NIAID)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

The LIR is currently studying prospectively the largest group of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic, and therapeutic results obtained over the past 17 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. In addition, we have described a new vasculitic syndrome which we have termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day CS in several, formerly universally fatal diseases such as Wegener's granulomatosis. In this regard, we are now following over 120 patients with Wegener's granulomatosis in which we demonstrated a 93% remission and cure rate. We have now applied these approaches with remarkable success to other vasculitic syndromes such as systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and lymphomatoid granulomatosis. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of lymphocyte activation and immunoregulation seen in these diseases. In addition, the precise effects of various therapeutic regimens, particularly CS and cytotoxic agents, on human lymphoid cells have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function. (Fauci, Leavitt, Volkman, Lane, Rook, Gocke, LIR/NIAID; Parrillo, CCM/CC; Cupps, Georgetown University)

Studies in Autoimmune Thyroid Disease

Over the past year, the LIR conducted studies aimed at delineating the immunopathogenic mechanisms involved in Graves' disease and Hashimoto's thyroiditis. Major histocompatibility complex (MHC) class II antigen expression by thyroid follicular cells was studied and it was demonstrated that these cells can function as antigen-presenting cells (APC) in both diseases. It was demonstrated that these APC present surface autoantigens such as thyroglobulin and microsomes to T cells by virtue of their MHC class II antigen expression. We demonstrated that recombinant γ-IFN, but not α-IFN or IL-2, induced 80-100% of thyroid follicular cells to express HLA-DR and about 50% to express HLA-DQ, strongly suggesting that IFN released during a viral infection or an ongoing immune response may contribute to the development of autoimmune thyroid disease in susceptible individuals by promoting the necessary conditions for the presentation of thyroid autoantigens to reactive T cells. Furthermore, by employing cell cloning

technology, we demonstrated that the thyroid gland is infiltrated with autoreactive T cells in Graves' disease and Hashimoto's thyroiditis and that these T cells can be expanded in vitro by IL-2. We also demonstrated that these clones proliferate in response to MHC class II antigen-positive thyroid cells, providing compelling evidence that these latter cells initiate or perpetuate the autoimmune process. (Weetman, Margolick, Fauci, LIR/NIAID; Weintraub, NIADDK)

Studies in Other Immune-Mediated Diseases

In 1983-1984 we identified a phagocytosis-inducing factor derived from the lymphocytes of patients with erythrophagocytosis syndromes and angiocentric lymphoproliferative diseases as well as normals. Over the past year, we have biochemically characterized the factor and have identified the T4 cell as its cell of origin. We are currently in the process of purifying this factor with the plan to clone the genes for this novel lymphokine.

We had previously demonstrated that patients with the Chediak-Higashi syndrome manifested a selective defect in their NK cell function. This was the first demonstration in man of a selective defect of NK cells. Over the past year, we demonstrated that this defect can be corrected in vitro by IL-2. This observation has important potential therapeutic implications in this disease which is characterized by the development of malignant lymphoproliferative disease.

We have continued our clinical and pathogenic studies on the idiopathic hypereosinophilic syndrome and have further characterized the specific components of the human eosinophil which play major roles in the pathogenesis of the syndrome. Finally, we are continuing our studies on the natural history, immunopathogenesis, and therapy of idiopathic dilated cardiomyopathy. The therapeutic trial should be completed in 1986, and preliminary results indicate that CS are effective in improving the underlying disease process. (Margolick, Rook, Fauci, LIR/NIAID; Parrillo, CCM/CC; Jaffe, LP/NCI; Henderson, University of Washington; Gleich, Mayo Clinic)

## Future Plans and Objectives

Over the past year, the LIR has made significant advances in the identification, isolation, purification, and characterization of factors involved in the activation, proliferation, and differentiation of human B lymphocytes. Now that we have these factors available in highly purified form, we will direct our efforts at identifying and characterizing the cellular receptors for these factors in order to more precisely delineate the mechanisms of immunoregulation of the human B cell cycle. We will pursue our studies utilizing our monoclonal antibody directed against HWM-BCGF not only for the purposes of cloning the appropriate genes but also to further investigate the physiologic roles of BCGF.

Our observation that certain subsets of B cells produce while others respond to BCGF will be pursued, particularly in attempting to extrapolate this finding to the T cell-independent expansion of the human B cell repertoire as well as to an understanding of the role of BCGF autocrine function in the development of certain B cell malignancies.

We will pursue the finding that IL-2 and γ-IFN synergize in the induction of differentiation of activated B cells by employing molecular biologic techniques to dissect the precise mechanisms of this synergy.

We have been able to utilize the model system of HTLV-I infection of human antigen-specific T cell clones and B cells to dissect out the functions of certain lymphocyte subsets. In addition, we have studied the functional capabilities of phenotypically defined T cell clones. We will actively pursue studies in these areas, particularly those directed at further delineating the functional subsets of T cells responsible for the regulation of B cell cycle events via secretion of B cell tropic factors.

We will continue our very fruitful studies on the pharmacologic modulation of the human immune response by further pursuing the mechanisms of action whereby CS, CsA, TGF-β, ACTH, and β endorphin affect the immune response in general and the events of the human B cell cycle in particular.

Studies on the molecular biologic approach to immunoregulation will continue with expanded studies on the molecular events associated with the induction of the c-myc oncogene as well as the IL-2 growth factor and IL-2 receptor genes. A major effort will be undertaken towards cloning the genes for BCGF and BCDF.

The LIR has a major committment to the study of the clinical aspects, virology, molecular biology, immunology, specific therapy, and immunologic reconstitution in AIDS. We plan to continue the intensity of this effort, which over the past year has resulted in several major advances. We will pursue our studies on the immunopathogenesis of the syndrome, relying heavily on our capability to probe at the molecular level the effects of the virus on the target T4 cells. Of particular interest and importance is the observation made by our laboratory over the past year that the virus can persist in a latent form in infected T4+/Leu-3+ cells. The relationship between persistent viral infection and the functional capability of the latently infected T4 cell will be actively pursued. We will use our infectious clone to pursue studies on the pathophysiology of the AIDS retrovirus. In addition, we will utilize

our recently developed in situ hybridization techniques to study the scope of virus infection in different body compartments and various lymphocyte subsets. The fact that we now have DNA clones which have been expressed in prokaryotic systems will provide us with viral protein of highest purity which will be utilized to delineate the precise nature of the immune response to the AIDS retrovirus. These studies will have obvious important implications in development of effective vaccines. We now have monoclonal antibodies directed against envelope glycoproteins of the virus. We will utilize these antibodies to block in vitro the activity of the virus in experiments aimed at ultimately employing these reagents in vivo for the purposes of specific blocking of viral intercellular transmission and elimination of infected cells which express the viral envelope proteins on their cell surface. All of our ongoing studies involving therapeutic trials of agents directed against the opportunistic infections as well as the AIDS retrovirus itself will continue. Of particular importance is the expanded clinical trial of suramin, since phase I clinical trials have yielded encouraging results. We have already embarked on the initial phases of the bone marrow transplantation studies combining suramin therapy with cellular reconstitution. This line of study will be intensively pursued.

Our large scale clinical and immunopathogenic studies on the spectrum of vasculitic syndromes, which have proved so fruitful from a clinical and immunopathogenic standpoint, will continue as will our studies on the idiopathic hypereosinophilic syndrome and idiopathic dilated cardiomyopathy.

## Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established in late 1980 and is now 5 years old. The theme of the LIR is the study of the mechanisms of activation and immunoregulation of human immunocompetent cells, particularly B lymphocytes, in normal individuals and in a variety of disease states characterized by abnormalities of immune function. In addition to basic research, the LIR continues to conduct the major portion of the clinical studies which are carried out in the NIAID Intramural Program within the Clinical Center.

Over the past year, there have been some important administrative changes which have had an impact on the LIR. Dr. Anthony S. Fauci, Chief, LIR, was appointed Director, NIAID, in November 1984. He will continue with dual appointments as Chief, LIR, and Director, NIAID. Dr. H. Clifford Lane was granted tenure this year, being the second tenured person in the LIR together with Dr. Fauci. Dr. Lane was also appointed Deputy Clinical Director, NIAID, and will assume increasing responsibility for the clinical and administrative matters within the LIR, particularly in the area of AIDS research. Dr. Randi Y. Leavitt has assumed a major role in assisting with clinical activities related to the LIR and will remain on as a Senior Staff Fellow following completion of her Medical Staff Fellowship. Drs. Julian L. Ambrus, Jr., and Debra L. Bowen have completed their Medical Staff Fellowship and will remain in the LIR as Senior Staff Fellows. Dr. Ambrus will be put on a tenure tract with the plan of converting him to a Senior Investigator. Dr. John H. Kehrl was appointed a Senior Investigator last year and will continue on in that capacity. Dr. Ulrich Siebenlist joined the LIR last year to head a molecular biology component of the laboratory. Over the past year, Drs. Peter Zipfel and Mark Brunvand, Guest Researchers; Dr. Peter Bressler, a first year Medical Staff Fellow; and Ms. Nicola Salvatore, a biologist, joined Dr. Siebenlist's group. The second Medical Staff Fellow to join the LIR in 1984-1985 is Dr. Scott Koenig, who is working with Dr. Lane on the AIDS project. Dr. Harris Goldstein entered his third year of Medical Staff Fellowship this year. Three Visiting Fellows from Japan, Drs. Shoken Tomita, Toshimasa Nakagawa, and Naoko Nakagawa, joined the LIR this past summer. They will continue on for a 2- to 3-year fellowship. Dr. Alain H. Rook, a Commissioned Officer in the USPHS, transfered from the FDA to the LIR this past summer and will be performing studies on the cytotoxic capability of lymphoid cells from AIDS patients.

When Dr. Kenneth W. Sell, Scientific Director, NIAID, left the Institute, there were a number of individuals under his laboratory supervision who were working directly on AIDS-related projects. Since the LIR and the LMM were the only intramural laboratories which were heavily involved in AIDS research, these individuals were reassigned to those two laboratories. In this regard, joining the LIR were biologists Mr. J. Shawn Justement, Ms. Sarah B. McCoy, Mr. Douglas Powell, Ms. Esther Racoosin, and Ms. Audrey Kinter; Experts Drs. Henry L. Francis and Thomas M. Folks; and Staff Fellow Dr. Marilyn M. Lightfoote. Medical Officer Dr. Thomas Quinn, who directs the Institute's Zairean AIDS project, also transfered to the LIR. These reassignments resulted in a much-needed consolidation and coordination of intramural AIDS projects.

Leaving the LIR this year are Dr. David J. Volkman, who will join the Department of Medicine at the State University of New York at Stony Brook;

Dr. Joseph B. Margolick, who will join the Department of Medicine at Johns Hopkins School of Medicine; Dr. Anthony P. Weetman, who will complete his Visiting Fellowship and return to England to assume a faculty position at Hammersmith Hospital in London; Dr. David J. Gocke, who finished his sabbatical year in the LIR and returned to his position as Professor of Medicine and Chief of the Division of Allergy, Immunology, and Rheumatology at Rutgers School of Medicine; and Dr. Lê thi Bich-Thuy, who finished her Visiting Fellowship and joined Dr. Queen of the NCI for an additional fellowship.

Ms. Lucy Renzi was reassigned to the position of Secretary to the Director, NIAID, and was replaced in the LIR by Ms. Joan Eccard, who assumed the position as Lab Chief's secretary; Ms. Ann C. London was reassigned from her position as Editorial Assistant, LIR, to the position of Editorial Assistant, OD, NIAID.

The laboratory space remains consolidated in the B wing of the 11th floor of the Clinical Center.

## Honors, Awards, and Scientific Recognition

Over the past year, members of the LIR, particularly in the persons of Drs. Anthony S. Fauci and H. Clifford Lane, have received a number of awards and honors. In November 1984 Dr. Fauci was appointed Director, NIAID. In early 1985, Dr. Lane was appointed Deputy Clinical Director, NIAID; in addition, Dr. Lane was granted tenure by the NIH Tenure Committee.

Dr. Fauci continues to serve on a number of committees of scientific importance. Over the past year, he was appointed to the Albert Lasker Medical Research Award Jury. He also enters the second year of his term as a member of the Board of Directors of the American Board of Allergy and Immunology. This year, he served as the Chairman of the Search Committee for the Director, National Institute of Child Health and Human Development. He was made a member of the National Diabetes Advisory Board, the National Digestive Diseases Advisory Board, the Department of Health and Human Services (DHHS) Task Force on Alzheimer's Disease and the DHHS Committee to Coordinate Environmental and Related Programs.

Dr. Fauci serves on a number of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He remains Associate Editor in charge of allergy and immunology of the American Journal of Medicine. He currently maintains his position on the Editorial Boards of Clinics in Immunology and Allergy, The Annals of Allergy, The Journal of Immunopharmacology, EOS, Clinical and Experimental Rheumatology, La Ricerca, Clinical Immunology and Immunopathology, Immunologia Clinica e Sperimentale, Physicians' Journal Update, Immunopharmacology, the Journal of Molecular and Cellular Immunology, and Cellular Immunology. Over the past year, Dr. Fauci was also appointed as the Advisory Editor for North America for the journal Thymus. In addition, he was appointed to the Advisory Board of the Journal of Clincal Immunology. He continues to co-edit with Dr. John I. Gallin the book series ADVANCES IN HOST DEFENSE MECHANISMS. This year he edited the textbook CURRENT THERAPY IN ALLERGY, IMMUNOLOGY, AND RHEUMATOLOGY, and he is also a co-editor of the textbook CURRENT THERAPY IN INTERNAL MEDICINE. Of note is the fact that over the past year, Dr. Fauci was appointed as an editor of HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. He will be responsible for editing the immunology, rheumatology, and allergy sections of that book as well as portions of the infectious diseases and oncology sections. Finally, Dr. Fauci has contributed a number of invited chapters covering a variety of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases. Over this past year, Dr. Lane has been asked to contribute chapters to major textbooks of medicine and medical subspecialties.

As part of the recognition for scientific accomplishments, clinical investigators may be asked to visit outside institutions and serve for periods of from 2 to 5 days as Visiting Professor within a given institution. In this regard, Dr. Fauci has been asked to and did serve as Visiting Professor at several major institutions throughout the year. Among these were three prestigious Visiting Professorships: The George Thorn Visiting Professorship at the Brigham and Women's Hospital and the Harvard Medical School; Visiting Professor of Medicine at Tufts-New England Medical Center; and Visiting Professor of Medicine at Baylor University College of Medicine. Dr. Lane was a Visiting Professor at West Pennsylvania Hospital.

In addition, Dr. Fauci was asked to give several major or named lectureships during the year. He delivered the Taft B. Schreiber Lecture at the Cedars-Sinai Hospital in Los Angeles. He was an invited symposium speaker at the Sixteenth International Leucocyte Culture Conference in Cambridge, England. He was an invited symposium speaker at the 6th European Congress of Immunology in Interlaken, Switzerland. In addition, he was an invited symposium speaker at the Fundamental Aspects of Rheumatology Symposium in Stockholm, Sweden, and the 10th International Conference on Sarcoidosis and Other Granulomatous Disorders. He was an invited symposium speaker at the Second International Workshop on Human Leukocyte Differentiation Antigens and the Interscience Conference on Antimicrobial Agents and Chemotherapy. Dr. Fauci was an invited plenary lecturer at the Infectious Diseases Society of America meeting. He delivered a State-of-the-Art lecture and moderated a panel at the American College of Physicians Southeastern States Scientific Meeting. He was an invited symposium speaker at the 2nd William B. Castle Symposium on Advances in Hematology and the American Academy of Dermatology 43rd Annual Meeting. In addition, he was an invited symposium speaker and chairperson of a scientific session at the Fourth International Symposium on Hemophilia Treatment. Dr. Fauci delivered a State-of-the-Art lecture at the 66th Annual Session of the American College of Physicians. He delivered the Eighth Annual Louis Weinstein Lecture at the Tufts-New England Medical Center. He was an invited plenary lecturer and chaired a session at the symposium on Recent Advances in Primary and Acquired Immunodeficiencies. He delivered the 1985 Memorial Lecture of the American Gastroenterological Association. He was an invited plenary speaker at the Sixty-Third Annual Meeting of the American College Health Association and was an invited symposium speaker at the 1985 Annual Meeting of the American Association for the Advancement of Science. In addition, Dr. Fauci delivered the prestigious George Thorn Lecture at the Brigham and Women's Hospital and Harvard Medical School.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER: Z01 AI 00210-05 LIR

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Anthony S. Fauci | Chief | LIR, NIAID |
| Others: | David J. Volkman | Senior Investigator | LIR, NIAID |
| | John H. Kehrl | Clinical Associate | LIR, NIAID |
| | Julian L. Ambrus, Jr. | Senior Staff Fellow | LIR, NIAID |
| | Harris Goldstein | Medical Staff Fellow | LIR, NIAID |
| | Joseph B. Margolick | Medical Staff Fellow | LIR, NIAID |

COOPERATING UNITS (if any)
MB, NCI, W. Greene; LCI, NIAID, E. Brown, A. Tenner; LCP, NCI, M. Sporn; LPD, NIAID, T. Nutman, E. Ottesen; LTCB, NCI, M. Popovic, R. C. Gallo

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 12 | 9 | 3 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanisms of activation, proliferation, and differentiation of human B cells were studied. Particular attention was paid to the factors operative in the B cell cycle. A high molecular weight B cell growth factor (HMW-BCGF) was identified, isolated, characterized, and purified to homogeneity. Its specific binding to activated B cells was demonstrated. A monoclonal antibody directed against HMW-BCGF was produced which specifically inhibited the activity of the factor, specifically bound to the factor, and specifically absorbed out the factor from culture supernatants. The production of BCGF by normal B cells and malignant B cell lines was demonstrated suggesting an autocrine function for this factor. Interleukin-2 (IL-2) receptors were demonstrated on activated B cells and IL-2 was demonstrated to play a major role in the proliferation and differentiation of human B cells. γ-Interferon synergized with IL-2 in the induction of differentiation of B cells. The phenotypic expression of certain T cell clones were correlated with defined functional capabilities of the clone in the regulation of B cell function, particularly via production of B cell differentiation factor (BCDF). Human T cell clones and normal B cells were transformed with human T lymphotropic virus (HTLV-I) and used as a model system for the delineation of certain lymphoid cell functions such as the expression of IL-2 receptors and the response to IL-2. The pharmacologic modulation of the human immune response was studied particularly with regard to the effects of corticosteroids, cyclosporin A, transforming growth factor-β, and certain neuropeptides on certain distinct phases of the human B cell cycle.



PHS 6040 (Rev. 1/84) GPO 914-918

| | | | |
|---|---|---|---|
| Others: | H. Clifford Lane | Senior Staff Fellow | LIR, NIAID |
| | Debra L. Bowen | Medical Staff Fellow | LIR, NIAID |
| | Shoken Tomita | Visiting Fellow | LIR, NIAID |
| | Naoko Nakagawa | Visiting Fellow | LIR, NIAID |
| | Toshimasa Nakagawa | Visiting Fellow | LIR, NIAID |
| | Lê thi Bich-Thuy | Visiting Fellow | LIR, NIAID |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00211-04 LIR

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Human Lymphocyte Subsets Employing Cloning and Hybridoma Technology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Anthony S. Fauci Chief LIR, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00212-05 LIR

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

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LAB/BRANCH

Laboratory of Immunoregulation

SECTION

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NIAID, NIH, Bethesda, Maryland 20205

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☒ (a) Human subjects x☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immunopathogenesis of a number of immune-mediated diseases and/or diseases characterized by abnormalities of immune function was investigated. Major histocompatability complex (MHC) class II antigen expression by thyroid follicular cells was studied which demonstrated that these cells can function as antigen-presenting cells (APC) in Graves' disease (GD) and Hashimoto's thyroiditis (HT). These APC present surface autoantigens such as thyroglobulin (Tg) and microsomes (M) to T cells by virtue of their class II antigen expression. We demonstrated that recombinant γ-interferon (IFN) but not α-IFN or interleukin-2 (IL-2) induced 80-100% thyroid follicular cells to express HLA-DR and about 50% to express HLA-DQ, strongly suggesting that IFN released during a viral infection or an ongoing immune response may contribute to the development of autoimmune thyroid disease in susceptible individuals. Using cell cloning technology, we demonstrated that the thyroid is infiltrated with autoreactive T cells in GD and HT and that these T cells can be expanded in vitro by IL-2. The proliferation of these clones in response to MHC Class II antigen-positive thyroid cells provides compelling evidence that these latter cells initiate or perpetuate the autoimmune process. Phagocytosis-inducing factor derived from patients with erythrophagocytosis and angiocentric lymphoproliferative disease as well as normals was biochemically characterized and its precise cell of origin was delineated. The natural killer cell defect in the Chediak-Higashi syndrome was corrected in vitro by IL-2. This has important potential therapeutic implications in this disease which is characterized by the development of lymphomas. We further characterized the components of the human eosinophil which play major roles in the pathogenesis of the idiopathic hypereosinophilic syndrome. We are continuing studies on the natural history, immunopathogenesis, and therapy of idiopathic dilated cardiomyopathy.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00213-05 LIR

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
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| Others: | David J. Volkman | Senior Investigator | LIR, NIAID |
| | H. Clifford Lane | Senior Investigator | LIR, NIAID |
| | Randi Y. Leavitt | Senior Staff fellow | LIR, NIAID |
| | Alain H. Rook | Senior Investigator | LIR, NIAID |
| | David J. Gocke | Guest Researcher | LIR, NIAID |

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Vasculitis is a clinicopathologic process characterized by inflammation of and damage to blood vessels. This process may result in a number of diverse clinical syndromes. The LIR is currently studying prospectively the largest group of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiologic, immunopathogenic and therapeutic results obtained over the past 17 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. In addition, we have described a new vasculitis syndrome which we have termed the polyangiitis overlap syndrome. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day corticosteroids in several, formerly universally fatal diseases such as Wegener's granulomatosis. In this regard, we are now following over 120 patients with Wegener's granulomatosis in which we demonstrated a 93% remission and cure rate. We have now applied these approaches with remarkable success to other of the vasculitic syndromes such as systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, the acute vasculitis of Sjögren's syndrome, and lymphomatoid granulomatosis. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of lymphocyte activation and immunoregulation seen in these diseases. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cells have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00390-02 LIR

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*

Studies of the Acquired Immunodeficiency Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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| Others: | Anthony S. Fauci | Chief | LIR, NIAID |
| | Thomas Folks | Expert | LIR, NIAID |
| | Alain H. Rook | Senior Investigator | LIR, NIAID |
| | Scott Koenig | Medical Staff Fellow | LIR, NIAID |
| | Marilyn M. Lightfoote | Staff Fellow | LIR, NIAID |

COOPERATING UNITS *(if any)*

CCM, CC, H. Masur, H. Alter, D. Henderson; COP, NCI, S. Broder, E. Gelmann; LMM, NIAID, M. Martin, A. Rabson, S. Benn, H. Gendleman

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An intensive effort was directed at studying the epidemiologic, virologic, immunologic, and clinical aspects of the acquired immunodeficiency syndrome (AIDS) and related illnesses. Over 300 patients with these diseases have been enrolled in research programs at the NIH. A study utilizing 500 hospital employees demonstrated the low risk of infectivity with the AIDS virus among hospital personnel. Studies of family groups revealed spread of infection only among sexual contacts within those groups. A cell line was developed which, following infection with the AIDS virus, gave rise to a cell line with latent AIDS virus. This latent virus could be induced with a variety of viral promoting agents including radiation and IUDR. Utilizing the technique of in situ hybridizaton, virus-positive cells were detected in peripheral blood, bone marrow, and semen of patients. Coculture experiments revealed that in peripheral blood the AIDS virus was contained within the T4 population. Monoclonal antibodies directed toward the envelope of the virus were developed in an effort to produce strategies to block the infectivity of the AIDS virus. The earliest critical abnormality of immunologic function noted in patients with AIDS or related illnesses or other forms of AIDS virus infection was a profound inability to respond to soluble protein antigen. Clinical trials of recombinant γ-inteferon were unsuccessful in attempting to alter the clinical course of patients with AIDS but did demonstrate that the drug had potent immunomodulatory properties when given to humans. Recombinant interleukin-2 was found to enhance immune function and decrease tumor size in patients with AIDS and early Kaposi's sarcoma. Two antivirals directed against AIDS virus, suramin and ribavirin and one, dihydroxypropoxymethylguanine, directed against a major infectious complications of AIDS (cytomegalovirus), were entered into initial clinical trials.



PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00431-01 LIR |
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PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Biologic Approach to the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
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| | Peter Zipfel | Guest Researcher | LIR, NIAID |
| | Anthony S. Fauci. | Chief | LIR, NIAID |
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IB, NCI, K. Kelly; MB, NCI, W. Greene, W. Leonard; PD, NCI, N. Holbrook; Stanford University, J. Crabtree

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The activation process of T lymphocytes was characterized from an intracellular, molecular point of view. The c-myc oncogene, the interleukin-2 (IL-2) growth factor and the IL-2 receptor gene are known to be induced during the early activation of T cells. We have determined regulatory regions within the chromatin structure of these genes, which will allow us to dissect the nuclear molecular events resulting from the transmission of the extracellular activation signal. Along the same lines, a model system has been developed to study the effect of differentiation at the nuclear level. The promyelocytic leukemia cell line HL60 can be differentiated terminally in vitro. Concomitantly, the c-myc oncogene is downregulated and its chromatin changes dramatically. We have initiated also more broadly based studies directed at the activation process of T cells. cDNA libraries were made from activated peripheral blood T cells in order to identify genes which are specifically and immediately induced upon mitogenic stimulation. A similar approach has been utilized in order to identify the genes coding for the lymphokines which are elaborated by these T cells after stimulation and in particular to identify the gene for the well-characterized B cell growth factor (BCGF). Gene cloning of this factor has been pursued also by preparing distinct cDNA libraries from a B cell tumor which expresses it constitutively. These libraries allow screening for the growth factor by two methods: one is based on partial amino acid sequence information of the purified growth factor, and the other method is based on the detection of BCGF by polyclonal antibodies in an expression library.

LABORATORY OF INFECTIOUS DISEASES
1985 ANNUAL REPORT

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National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

The mission of the LID continues to be definition of the cause and epidemiology of medically important virus diseases and development of means for their control. The activities of the laboratory span a wide range of scientific inquiry from identification and characterization of viruses that cause acute disease of the respiratory and gastrointestinal tracts and the liver to basic molecular studies of virus structure, function and genome organization. The techniques of molecular biology and immunology are employed to probe pathogenesis and to develop purified subunit antigens and attenuated virus mutants for use in prevention of infection and disease by the major viral pathogens of the respiratory and gastrointestinal tracts and the liver.

## Hepatitis A Virus

Attenuation The hepatitis A virus (HAV) is a picornavirus that was isolated in tissue culture for the first time relatively recently. We have succeeded in isolating virus directly from human clinical materials without the intervening serial passage in marmoset monkeys that was necessary for its initial isolation in tissue culture. We have used as cell substrate primary African green monkey kidney (AGMK) cells, a cell type approved for vaccine development. Over 30 serial passages of the HM-175 strain of HAV, obtained from Dr. Ian Gust (Melbourne, Australia), have been achieved in AGMK cells. Initially, the virus grew very slowly and to low titer ($10^5$), but with passage replication became more rapid and more virus was produced ($10^8$). Nonetheless, even at the highest passage level HAV remained strongly cell-associated. The tissue culture-adapted HAV mutant was clearly attenuated for chimpanzees compared to the parental virus, which is highly virulent for this species. Tissue culture-adapted HAV from passage level 10, 20 or 30 infected champanzees and induced a high serum antibody response, but there was little or no histological or biochemical evidence of liver disease. Stability of the attenuated HAV was demonstrated by passaging the virus 3 times serially in chimpanzees. During the third passage the virus retained its attenuation for chimpanzees. Most of the chimpanzees which had previously been infected with attenuated HAV (passages 10 and 20) were resistant to challenge with the virulent parental virus. In contrast to the response of chimpanzees, marmosets inoculated with the tissue culture mutant of HAV developed significant enzyme elevations. Thus, the attenuation of HAV appears to be species specific. This makes it difficult to predict the response of humans to the HAV mutant. This assessment awaits carefully controlled studies in adult volunteers (Feinstone, Purcell).

A rapidly growing HAV mutant has also been used to develop a neutralization test which should facilitate vaccine development as well as analysis of monoclonal antibodies (Feinstone).

Molecular cloning of HAV genome. The molecular cloning of HAV genomic sequences in *E. coli* was achieved previously using RNA extracted from purified virions. Virion RNA served as a template for synthesis of double-stranded complementary DNA (cDNA) which was then inserted into a plasmid vector (pBR322) and biologically amplified in *E. coli*. Analysis of restriction digests,

hybridization reactions, and DNA sequence yielded a map of overlapping cDNA clones spanning approximately 7500 nucleotides, which represented about 99% of the HAV genome. Primer extension was also used to prepare cDNA from the 5' terminus of the HAV genome for molecular cloning. One of several candidate 5' clones yielded a terminal sequence of 22 nucleotides which completes our sequence of the genome at its 5' terminus (Ticehurst, Baroudy, Purcell). The sequence of two large regions of the genome has been determined. A sequence of 3119 bases corresponding to the 5' end of HAV RNA contains an open reading frame that begins ~750 bases from the 5' terminus and extends 2407 bases which is as far as analysis has been completed. Sequence preceding this major reading frame has nine other potential initiation sites, but the longest peptide that can be translated in this region is only 26 amino acids. This pattern is consistent with the genomic organization of other picornaviruses. The entire sequence of a clone that mapped to the 3' end of HAV RNA has also been determined. A poly(A) tract of 15 bases was found at one end of this clone thus orienting the 3' end of the genome. This poly (A) tract is 51 bases downstream from two closely spaced termination codons that are preceded by 1407 bases in an open reading frame that is presumably continuous with that present at the 5' end of the genome (Baroudy, Ticehurst). These sequenced regions of the HAV genome have been compared, using computer programs, to analogous regions previously determined for other picornaviruses and HAV was found to be quite distinct (Ticehurst, Baroudy). In the past year we have sequenced sufficient clones to span 7.0kb of the 7.5kb HAV genome. Morever, it was possible to deduce from this data the putative sequence of VPg, a protein believed to be covalently attached to the 5' end of the genome. In collaboration with Dr. Maloy (LIG), a peptide corresponding to the carboxy end of this protein was synthesized and antisera were prepared that immunoprecipitated the VPg-RNA genome complex. Other peptides have been synthesized based upon predicted HAV amino acid sequence of capsid proteins. Antiserum prepared against a peptide from the VP1 capsid protein neutralized HAV infectivity in tissue culture (Baroudy, Ticehurst).

Cloned cDNA has also been used as a hybridization probe for detection of HAV RNA in tissue culture and in serum, and fecal specimens. Hybridization with nick-translated cDNA was more sensitive for detection of HAV than radioimmunoassay of HAV proteins. HAV RNA was detected for a longer period than antigen when serial stool samples from several HAV outbreaks were analyzed (Ticehurst).

Attempt to prepare full-length infectious HAV cDNA. Because the HAV genome contains RNA it is highly unlikely that site-specific mutagenesis can be accomplished unless HAV genetic information is transferred into a DNA form that is infectious. Recently this was accomplished for poliovirus, another picornavirus whose genomic organization is similar to HAV. Full length poliovirus cDNA is infectious when inoculated into permissive tissue culture cells. A similar approach has been initiated with HAV in which our goal is to construct a full length infectious HAV cDNA and use it for site-specific mutagenesis. A set of six cDNA clones that span the entire genome of HAV were ligated together, in stepwise fashion, to create a successively larger sequence that ultimately included the entire HAV genome. A single clone was constructed in pBR322 which was thought to contain the entire HAV genome as a cDNA analog. The putative full length HAV cDNA, was excised from pBR322 and inserted into an SV40 vector (containing the SV40 early and late promoters, enhancer sequences, and origin of replication). Transfection of tissue culture cells (in vitro)

and marmosets (by intrahepatic inoculation) with this vector failed to generate HAV. Concurrently, transfection of the same type of tissue culture cells with full length poliovirus cDNA yielded infectious poliovirus. Fine structure mapping of the putative full length HAV cDNA showed that about 40 base pairs had been deleted during the ligation process. Currently, construction of a full length infectious HAV cDNA is in progress (Cohen, Ticehurst, Baroudy).

## Hepatitis B Virus

Clinical and experimental studies of hepatitis B vaccines. Hepatitis B virus (HBV) is a unique 42nm virus of complex structure that contains a double-stranded circular DNA with a single-stranded gap spanning 20-50 percent of the genome. It is the first recognized member of what is now a small group of viruses designated the "hepadnaviruses". HBV causes considerable morbidity and mortality, accounting for 30-50 percent of clinical hepatitis diagnosed in the U.S. and most developed countries. However, it is in Asia and Africa that the virus has its greatest impact. HBV causes a chronic infection, usually associated with hepatitis, in approximately five percent of the world's population, and this form of HBV infection may lead to death from chronic hepatitis, cirrhosis or hepatic cell carcinoma. Although relatively rare in developed countries, hepatic cell carcinoma is one of the leading causes of death from cancer in Africa and Asia, and there is considerable evidence that HBV is a causative factor in such cancer. For these reasons the control of HBV is an important public health goal.

Research in the Hepatitis Viruses Section as well as in academic and industrial laboratories elsewhere over the past decade has led to the recent development and licensing of hepatitis B vaccines. Such vaccines contain purified HBV surface antigen (HBs Ag) derived from the plasma of individuals chronically infected with HBV. These vaccines have been shown to be both safe and effective in preventing type B hepatitis.

Clinical testing and characterization of plasma-derived vaccines prepared by the NIAID have been completed. An alum-adjuvanted preparation was found to be highly immunogenic and well tolerated in adult volunteers. Sixty-eight percent of vaccinees developed antibody within one month of vaccination and 95 percent seroconverted following completion of the six-month vaccination schedule. Antibody persisted three to four years or more in some persons. The NIAID vaccine was also highly immunogenic and nonreactogenic in children and infants. The rapid antibody response to the NIAID vaccine suggested that it may be useful in preventing perinatal transmission of HBV from infected mothers to their offspring, an event that occurs frequently in Asia (Ticehurst, Purcell).

An efficacy trial in newborn infants whose mothers were persistently infected with HBV was conducted in the People's Republic of China between July 1982 and August 1984. In a double-blind randomized study, the NIAID vaccine was compared with a placebo, a vaccine prepared at the Beijing Institute of Vaccine and Sera (BVIS), and a combination of BIVS vaccine plus hepatitis B immune globulin (HBIG). The frequency of HBsAg at 6 months in offspring of HBeAg positive mothers was 75 percent. Despite this high frequency of transmission, the NIAID vaccine was approximately 90 percent effective in preventing HBV infection when compared to the experience of placebo recipients. Protection was measured by reduction in detection of HBsAg or anti-HBc; the

latter was assayed at 18 months of age. This level of protection compares very favorably with the best results obtained by others with HBV vaccine plus HBIG. The NIAID vaccine was also 89% effective in preventing the development of chronic hepatitis B by one year of age, similar to BIVS vaccine plus HBIG and more effective than BIVS vaccine alone. These results are encouraging and suggest that a potent hepatitis B vaccine, if administered within hours of birth, can prevent most maternal-fetal transmission of chronic HBV infection without the need for HBIG (Purcell).

Second and third-generation hepatitis B vaccines. Currently licensed HBV vaccines are very expensive, available in limited quantities, and, until recently, required safety-testing in chimpanzees, the only susceptible animal suitable for such tests. In addition, the recent discovery of acquired immunodefficiency syndrome (AIDS) and the similarity of its epidemiology to that of hepatitis B virus have raised questions about the safety of current hepatitis B vaccines since the HBsAg-containing plasma used for their manufacture is drawn from populations at risk of acquiring AIDS. It should be emphasized that there is no evidence that hepatitis B vaccine poses any risk to recipients, and vaccine recipients have not developed antibody to HTLV-III, but fear of possible transmission of AIDS has led to under-utilization of vaccine among high-risk populations who should be protected. Thus, there is a need for second-generation hepatitis B vaccines that will be readily available, economical and acceptable for wide-spread administration. A number of approaches to the development of such vaccines utilize, directly or indirectly, recombinant DNA technology.

An intriguing application of this new technology to the development of second-generation vaccines was recently initiated in collaboration with Dr. B. Moss of LVD. A 1350 bp fragment of HBV DNA that contains the coding sequence for HBsAg was used to construct a viable vaccinia-HBsAg recombinant. Intradermal infection of rabbits with the recombinant vaccinia virus stimulated the production of antibodies to HBsAg. Chimpanzees similarly infected with the recombinant vaccinia did not develop demonstrable anti-HBs antibodies but they were protected against hepatitis when subsquently challenged with live HBV. The chimpanzees developed high titers of anti-HBs at a time when a control chimpanzee first had detectable HBsAg, suggesting that they had been "primed" by infection with the recombinant vaccinia-HBsAg and developed an anamnestic antibody response to newly-synthesized HBsAg following challenge with live HBV. This experiment was repeated with two modifications. First, the recombinant vaccinia virus used for vaccination was derived from the licensed vaccine strain, rather than laboratory strain used in the first experiment. Second, the chimpanzees were revaccinated with recombinant vaccinia-HBsAg one month after the primary vaccination in order to "boost" the immune response, a procedure that yielded high antibody titers in small laboratory animals. The chimpanzees have been challenged with live HBV and are being followed for evidence of infection. Unfortunately, neither chimpanzee developed detectable anti-HBs after either vaccination. More efficient expression of the HBV surface antigen gene in vaccinia must be engineered before this approach will have practical application (Purcell, Feinstone).

Finally, DNA recombinant technology has permitted not only the molecular cloning of the entire HBV genome but also the determination of its entire nucleotide sequence. The S gene codes for 226 amino acids that are arranged in a series of alternating hydrophobic and hydrophilic regions. In collaborative

studies with Drs. R. Lerner and J. Gerin, synthetic peptides representing a portion of one of these hydrophilic regions of the HBsAg polypeptide were studied. Both linear and cyclic forms (prepared by oxidation of cysteines to form a disulfide bond) of one of the peptides were compared. A group-reactive specificity as well as a type-specific antigen (the d/y allele) was detected in this region of HBsAg. Peptides representing the predicted amino acid sequences for both a subtype adw and a subtype ayw antigen stimulated antibody of the appropriate subtype in rabbits. This subtype specificity appears to be an inherent characteristic of the amino acid sequence and may be defined by as few as two amino acid substitutions (at positions 131 and 134), but the group-reactive a specificity is probably steric in nature because it is destroyed by reduction and alkalation of the cyclic peptide. Immunization of chimpanzees with the linear peptide (amino acids 110-137) stimulated anti-HBs that was subtype specific and transient, apparently because it was exclusively of the IgM class. Nevertheless, immunized chimpanzees were partially or completely protected when challenged with live HBV. The mechanism of this protection in the absence of demonstrable serum antibody is not known. Additional studies in chimpanzees with linear and cyclic forms of peptide 110-137 and with a short form (aa 125-137) of the peptide that contains the amino acid substitutions defining subtype specificity were also performed. Partial or complete protection was again observed, especially with the cyclic form of the peptide, but a significant number of animals not protected by the synthetic peptides developed chronic B hepatitis following challenge with live HBV. Although the numbers are too small to be significant, these observations suggest that caution should be exercised in the use of such peptides and that additional studies be performed to confirm this phenomenon and elucidate its mechanism (Purcell).

Another region of the HBV genome may also play a role in immunity to HBV. The "pre-S" region, that immediately precedes the "S" (surface antigen) gene, codes for a series of peptides that form "fusion" proteins with the gene product of the S region. These fusion proteins, ranging in size from approximately 33 kd to 46 kd, are minority components of HBsAg but appear to be integral parts of the virion and at least some of them are present on the surface of the virion. The pre-S region may play a role in initiation of infection because one of its fusion proteins contains a receptor site for polymerized human serum albumin, and this receptor is thought to be bind HBV to albumin which then binds to albumin receptors on hepatocytes. Antibody to pre-S region epitopes is present in the serum of most individuals convalescing from HBV, and it is the first HBV antibody that develops in many infected individuals. Individuals vaccinated with the NIAID vaccine, but not the Merck, Sharp and Dohme vaccine, developed antibody to pre-S peptide. The Merck vaccine is treated with pepsin and this treatment probably removes the pre-S peptide from HBsAg. Because the NIAID vaccine was recently shown to be more effective in preventing maternal-infant transmission of hepatitis B virus than any of the other vaccines tested to date, it is possible that the pre-S region (or other as yet unidentified epitopes) may be important in stimulating maximum protection against HBV. In order to examine this phenomenon further, chimpanzees have been vaccinated with synthetic peptides representing a portion of the pre-S gene product. In this study, carried out collaboratively with Drs. R. Lerner and J. Gerin, the chimpanzees responded with rapid development of high titered antibody to the pre-S region peptide. This is in contrast to the poor antibody responses previously induced by synthetic peptides representing regions of the S gene product. These chimpanzees will be

challenged to determine if pre-S peptide alone can protect against HBV (Purcell).

## Woodchuck Hepatitis Virus (WHV)

The similarities between HBV and WHV (another hepadnavirus) coupled with the tendency of each virus to cause chronic hepatitis and hepatic cell carcinoma in their respective hosts, makes WHV and its host, the woodchuck, a particularly interesting model system. In collaboration with Dr. J. Gerin a "vaccine" prepared from WHsAg in a manner similar to the hepatitis B vaccine, was administered to selected newborn animals. Other animals received a placebo. Certain of these animals were also inoculated with live WHV at birth and other offspring were exposed under natural conditions to their WHV-positive mothers. The purpose of the study, which will last several years, is to determine whether vaccine can interrupt the perinatal transmission of this hepadnavirus in its natural host. Observations made during this study should allow us to predict the results of similar vaccine interruption trials with HBV vaccine in man. Our ultimate goal is to prevent human hepatic cell carcinoma (HCC) by prevention of chronic HBV infection of the newborn, however, the effectiveness of this approach to prevention of HCC in man will not be known for 20 to 40 years (the incubation period from infection to development of HCC). However, the incubation period to HCC in the woodchuck is only 2 to 5 years. Thus, prognostic information should be available from the woodchuck-WHV model system within the next few years. This information should prove to be useful in planning for future use of HBV vaccines in man.

Two WHV vaccines were tested and both were found to be safe (ie, they did not contain infectious WHV) and capable of stimulating antibody that protected against challenge with live WHV. The vaccines prevented WHV-associated hepatitis when given to newborn woodchucks which were simultaneously administered live WHV. In contrast, placebo recipients developed hepatitis when infected with WHV at birth. Although a proportion of the vaccinated woodchucks developed serologic evidence of infection, it was inapparent and consisted solely of seroconversion to anticore antibody. Thus, the woodchuck model predicts that vaccination of the newborn human with HBV vaccine will offer significant protection against perinatal transmission of HBV leading to chronic infection. Surprisingly, most of the WHV infections of newborn woodchucks did not become chronic. This was true both for parenterally infected animals (inoculated with infectious WHV within one day of birth) as well as for offspring of chronically infected mothers. Previously we observed 30% chronic infection with WHV among wild woodchucks trapped in Maryland and Pensylvannia and this suggested that perinatal transmission to young woodchucks was probably the mechanism of chronic infection in the wild. In contrast, only one woodchuck inoculated with $10^6$ infectious doses of WHV within one day of birth developed a chronic infection although all of the inoculated animals that did not receive vaccine were infected (Purcell).

In subsequent studies it was found that inoculating infant woodchucks with a high concentration of virus resulted in a much higher (approximately 30%) rate of chronic infection. Thus, the high dose inoculum mimics the carriage rate found in nature. Furthermore, most animals which became carriers as the result of experimental infection developed hepatocellular carcinoma in less then two years. These studies demonstrate the value of the woodchuck as an

animal model for the study of hepatitis B virus-like infection and carcinogenesis (Purcell).

## The Delta Agent

The delta agent is a transmissible hepatitis agent that is defective because it requires co-infection with hepatitis B virus for its synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBs Ag. In collaboration with Dr. J. Gerin (Georgetown University), a portion of the putative genome has been cloned. Attempts to hybridize HBV and the delta agent, using cloned cDNA probes of the two genomes, failed to reveal a relationship between these organisms.

Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood or blood products (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. Light and electronmicrographic changes similar to those seen during acute non-A, non-B hepatitis infection have been detected in such chimpanzees, suggesting that delta may share some characteristics with other non-A, non-B agents. The delta agent has also been experimentally transmitted to woodchucks chronically infected with WHV, a hepadnavirus similar to HBV. Recently, serologic studies of Yucpa Indians (Venezuela) indicated that delta agent was etiologically associated with severe and often fatal hepatitis in this population which has a high incidence of hepatitis B virus infection. Serologic evidence that the delta agent is associated with severe hepatitis in Brazil (Labrea fever) and Colombia (Santa Marta hepatitis) has also been obtained (Purcell).

## Non-A, Non-B Hepatitis Agents

The non-A, non-B hepatitis viruses constitute an important cause of sporadic hepatitis. These agents also represent a constant threat to the safety of blood and blood products. Non-A, non-B hepatitis viruses have not been detected by immunologic assay, but these agents can be transmitted experimentally to chimpanzees and marmosets which have proved useful in titrating and characterizing these viruses. For example, it was recently demonstrated that a high titered non-A, non-B virus contains essential lipids; this characteristic should prove useful in the classification as well as the control of non-A, non-B hepatitis. Non-A, Non-B hepatitis continues to be an enigma for workers in this field. Extensive efforts to find a virus or an antigen related to this disease have not been successful and many erroneous claims have been made. For example, recently it was reported by Seto et al. (Lancet, 1984) that the agent of non-A, non-B hepatitis was a retrovirus based on the detection of reverse transcriptase activity in the plasma of patients with this disease. Extensive efforts in LID failed to reveal detectable reverse transcriptase activity in plasma from patients with acute non-A, non-B hepatitis (Feinstone, Purcell).

Currently efforts are underway to clone the nucleic acid of non-A, non-B virus employing substractive hybridization of non-A, non-B cDNA (prepared from mRNA derived from a non-A, non-B chimpanzee liver) with cDNA from normal, hepatitis A and hepatitis B chimpanzee livers (Feinstone).

Inactivation of hepatitis viruses in pooled plasma derivatives   Viral hepatitis in recipients of blood products, especially pooled plasma derivatives, continues to be a major problem. Mandated screening of all plasma units for HBsAg has diminished, but not abolished type B hepatitis in hemophiliacs and other recipients of clotting factors but non-A, non-B hepatitis continues to be a major medical problem for these individuals. In addition, the recognition of acquired immune deficiency syndrome (AIDS) in increasing numbers of hemophiliacs and the detection of antibody to HTLV-III in 50% of such individuals adds new urgency to the development of methods that can eliminate hepatitis viruses and retroviruses from clotting factors without markedly diminishing the potency of these labile proteins.

A series of collaborative studies were designed to develop inactivation methods that are applicable to the large-scale production of pooled plasma derivatives. Two approaches were utilized. The first was incubation of clotting factors in the lyophilized state at elevated temperature. Commercial factor VIII (anti-hemophilic factor, AHF) was seeded with a measured quantity of hepatitis B virus, lyophilized and then incubated at 60°C for 30 hours. Chimpanzees were inoculated with the heat-treated AHF or sham-treated AHF that had been held at 4°. Surprisingly, HBV survived the heating procedure with no apparent loss in titer (Feinstone, Purcell).

The second approach to inactivation of hepatitis agents is based on a previous finding that both HBV and the Hutchinson strain of non-A, non-B virus contain essential lipids, i.e., they are inactivated by exposure to lipid solvents. Since the delta agent utilizes the lipoprotein coat of HBV, it is likely that this agent is also inactivated by lipid solvents. Aliquots of a commercial lot of AHF were experimentally contaminated with HBV, the delta agent or the Hutchinson strain of non-A, non-B hepatitis virus and lyophilized. The lyophilized preparations were subjected to prolonged extraction with dry chloroform which was then removed by evaporation. Two lipid-containing viruses, vaccinia and avian influenza virus, were used as external and internal controls, respectively, of the inactivation procedure. The non-A, non-B hepatitis virus was completely inactivated by this procedure but, surprisingly, the HBV and delta agent were not completely inactivated. In this experiment, inactivation of the control vaccinia and avian influenza viruses was also not complete. For this reason the chloroform inactivation procedure was reevaluated.

Because earlier experiments with dry chloroform demonstrated complete inactivation of control lipid-containing viruses, it was suspected that the chloroform used in those studies contained a small amount of water. The importance of water was confirmed in subsequent experiments in which chloroform partially or completely saturated with water was used for inactivation of control viruses. Partially saturated chloroform was found to be partially effective and fully saturated chloroform fully effective in inactivating control viruses, even though, saturated chloroform contained less than 1% water and could be handled as a single liquid phase. Inactivation experiments are currently being repeated with the various hepatitis viruses and HTLV-III, using water-saturated chloroform. This procedure, when applied to commercial lyophilized factor VIII, results in retention of 100% of the factor VIII activity. In contrast extraction of aqueous factor VIII with chloroform, produces significant destruction of factor VIII activity. The modified chloroform extraction precedure may have considerable utility for rendering a

variety of plasma derivatives free of hepatitis viruses and retroviruses (Feinstone, Purcell).

## Influenza A Virus

### Genetics of attenuation conferred by avian influenza A virus genes

Previously we demonstrated that avian influenza A viruses which are restricted in the lungs of primates can serve as donors of attenuating genes to human influenza A wild type viruses. Avian-human influenza A reassortant viruses were constructed that contained the hemagglutinin (HA) and neuraminidase (NA) genes of a human wild type influenza A virus while the six other ("internal") genes were derived from an avian influenza A donor virus. These reassortant viruses replicated efficiently in tissue culture at 42°C like their avian influenza A virus parent but were restricted in replication, ie, attenuated, in the respiratory tract of monkeys and volunteers. Three avian influenza A viruses that were restricted in their growth in the lower respiratory tract of primates were used as donors of their 6 "internal" genes to virus reassortants derived from 3 separate human influenza A wild type viruses, A/Washington/80 (H3N2), A/Korea/82 (H3N2) and A/California/78 (H1N1). Six such reassortants were evaluated in susceptible adult volunteers and in each instance a satisfactory level of attenuation was observed. Immunologic responses were induced by each reassortant and resistance to wild type virus was observed in the one instance in which challenge was performed (Murphy, Snyder, Chanock).

We previously demonstrated that the NP and M genes of the avian influenza A/Mallard/NY/6750/78 (H2N2) virus appear to play a major role in the host range restriction of this avian influenza virus and its reassortants in primates. Furthermore, the combination of the avian influenza A/Mallard/78 RNA 1 and NS genes also contributes to restriction of replication. The observed polygenic nature of attenuation was encouraging because it is unlikely that this many avian influenza genes could easily develop the number of mutations required to gain the degree of virulence for man ordinarily expressed by human influenza A viruses, especially since avian influenza reassortants produce a restricted infection in susceptible individuals. Analysis of the nucleotide sequence of the M1 and M2 cistrons of the influenza A/Mallard/78 M gene indicated that there was significant sequence divergence in the M gene of human influenza A viruses, such as A/Udorn/72 and avian influenza A viruses, such as A/Mallard/78. The difference in the M2 (86% amino acid homology) cistron was greater than that of the M1 cistron (96%). The sequence of the monocistronic NP gene of avian influenza A/Mallard/78 virus was also determined and analysis of various NP sequences indicated that there was a significant divergence between human and avian influenza NP genes. The amino acid sequence of the avian influenza A/Mallard/NY/78 NP differed by 9.6% from that of the human influenza A/Udorn/72 strain. Fifteen of the 498 amino acids were species specific, i.e., at each of the fifteen positions, each of the three avian influenza NP genes which had been sequenced by us and others coded for the same amino acid, while each of three human influenza A strains sequenced by us and others had a specific amino acid at that position that differed from that of the avian influenza A viruses. These results indicate that there are classes of human influenza and avian influenza M and NP genes that have evolved separately in the two species. In addition to the species-specific loci, there was considerable amino acid sequence divergence between human influenza and avian influenza M and NP genes. Thus, it is likely that more than one amino

acid difference in both the NP and M genes is responsible for host range restriction of avian-human influenza virus reassortants in primates (Buckler-White, Murphy).

The avian influenza A virus, A/Pintail/Alberta/119/79, and its six-gene avian-human influenza reassortants were also restricted in replication in the respiratory tract of squirrel monkeys. To investigate which of the A/Pintail/79 influenza A virus genes or combination of genes is responsible for diminished replication in the respiratory tract of primates, reassortant viruses were produced that contained human influenza A virus surface antigens (HA and NA) from the A/Washington/80 (H3N2) virus and one or more internal genes derived from the avian A/Pintail/Alberta/79 influenza virus. To date, single gene substitution reassortants containing an avian influenza M, NP, NS, or RNA1 gene have been isolated. The preliminary results from these studies indicate: (1) Certain of the reassortant viruses do not grow efficiently in canine MDCK cells but replicate efficiently in chick kidney tissue culture; a particular constellation of polymerase genes specifies this host range restriction. (2) The M gene of the A/Pintail/79 avian virus does not specify restricted virus replication in monkeys. (3) The RNA1 and NS genes each specify a level of replication in the upper and lower respiratory tract of the monkey that is intermediate between that of the two virus parents. These observations suggest that: (1) A specific constellation of avian-human influenza polymerase genes restricts replication in mammalian tissues but not in chick kidney tissue culture and this type of restriction may prove useful for generation of attenuated influenza A reassortant viruses for use in live attenuated vaccines. (2) The basis for attenuation of avian influenza viruses in primates may vary for different avian influenza viruses, ie, different "internal" genes may be responsible for restriction exhibited by different avian influenza strains. We are now attempting to identify as many diverse avian influenza A virus genes that specify growth restriction in primates as possible. This may allow us to construct composite avian influenza A donor viruses containing three or more host range genes, each of which can separately attenuate a human influenza A virus for man. It may be necessary to bring together genes such as the M and NP genes of the A/Mallard/78 strain and the RNA 1 and NS genes of the A/Pintail/79 to produce a composite avian influenza A donor virus that yields reassortants which exhibit the desired balance between attenuation and immunogenicity while maintaining maximal genetic stability (Snyder, Murphy).

Genetics of attenuation conferred by genes of cold-adapted (ca) donor virus. We sought to identify the genes of the influenza A/Ann Arbor/6/60 (H2N2) cold-adapted (ca) donor virus that attenuate human influenza A viruses by constructing a set of reassortant viruses that each contained a single gene from the ca donor that codes for a protein other than the surface glycoproteins ("internal gene"), while all other genes were derived from the human wild type influenza A/Korea/1/82 (H3N2) virus. The NP and NS single gene substitution reassortant viruses did not exhibit the ca or ts phenotype. When administered to ferrets both the NP and NS single gene reassortant viruses and the wild type virus were able to grow well in the upper and lower respiratory tract. In contrast, a reassortant virus containing 6 "internal" genes derived from the ca donor grews to low titer in the upper respiratory tract and failed to replicate in the lungs. These observations indicate that the NP and NS genes of the ca donor virus do not play a major role in the attenuation of reassortants derived from the influenza A/Ann Arbor/60 ca parent virus (Snyder, Murphy).

Single or double gene substitution reassortants that possess only one or two ca "internal" genes and 6 or 7 genes derived from the human influenza A/Washington/80 virus (H3N2) were also evaluated in susceptible volunteers in an effort to elucidate the genetic basis for attenuation of ca reassortant viruses for humans. A reassortant virus which contained the PA polymerase and M genes from the ca parent, while the other genes were derived from influenza A/Washington/897/80 (H3N2) wild type virus, was evaluated in 14 seronegative adult volunteers who were inoculated intranasally with $10^{7.0}$ $TCID_{50}$. Their response to infection was similar to that observed for reassortants containing all six internal genes derived from the ca parent virus. In the context of previous observations which indicate that the ca M gene is not critical to attenuation, the present findings suggest that the PA polymerase gene plays a major role in the attenuation of reassortant viruses derived from the ca donor virus (Snyder, Murphy).

Duration of immunity following administration of live attenuated cold-adapted reassortant virus or inactivated virus vaccine. The protective efficacy of live attenuated ca reassortant influenza A H3N2 or H1N1 virus vaccine against experimental challenge with homologous wild type virus seven months after vaccination was compared with the efficacy of licensed inactivated virus vaccine in a study involving 106 seronegative college students. The live attenuated virus vaccine provided as much protection against febrile or systemic illness as the inactivated vaccine. Vaccine efficacy measured by prevention of febrile or systemic illness was 100% for ca H3N2 vaccine, 83% for inactivated H3N2 vaccine; 77% for ca H1N1 vaccine; and 67% for inactivated H1N1 vaccine. Unlike vaccinees given inactivated H3N2 virus vaccine, the ca H3N2 virus vaccinees shed significantly less virus than control, unvaccinated volunteers. Protective efficacy observed seven months post-vaccination with the live ca H3N2 virus vaccine was compared with that observed one to two months following vaccination. One to two months after vaccination 81% of the live ca H3N2 virus vaccinees were protected against infection, whereas at seven months only 31% were protected. Furthermore, complete protection against upper respiratory tract illness was seen after one month, whereas only 50% protection was observed at seven months. This diminution of immunity in the upper respiratory tract correlated with a 3-fold drop in nasal wash IgA HA antibody level over the six month period (Murphy, Snyder).

## Respiratory Syncytial (RS) Virus

Molecular biology of RS virus. Human respiratory syncytial (RS) virus, an enveloped virus that contains a single negative-sense strand of genomic RNA, is the most important agent of pediatric respiratory tract disease. Previously, complete cDNAs and complete nucleotide sequences were obtained for nine of the ten known viral mRNAs. Recently, synthetic oligodeoxynucleotides were used to direct dideoxynucleotide sequencing of intergenic and flanking regions in the viral genome. The results confirmed the sequences of the gene termini obtained from the cDNAs, indicating that the nine viral mRNAs initiate with the conserved sequence 5' GGGGCAAAUA$^{A}_{U}$... and terminate with the conserved sequence 5' ... AGU$^{A}_{U}$ A(N)$_{1-4}$ - poly A. Comparison of the intergenic and flanking sequences with the complete mRNA sequences established unambiguously the 3' to 5' order of the nine genes on the viral genome. Each gene was immediately followed (in genome-sense) by an oligo U tract of 4-7 residues that probably direct synthesis of poly A tails of the mRNAs by a reiterative copying mechanism. The intergenic regions varied in length from 1 to 52 nucleotides

and did not display obvious sequence conservation except that in all cases the last nucleotide (in genome-sense) was an A residue. Results to date confirm the initial observations concerning the complexity of the genetic map. The RS viral genome is different from other paramyxoviruses in (1) gene number (2) gene order, and (3) the variability in length and lack of sequence conservation of intergenic regions. Other less striking differences include the observation that the RS viral oligo U tracts are variable in length and that the gene sequences immediately adjacent to the oligo U tracts are not conserved. All of these differences are suggestive of a considerable evolutionary distance between RS virus and other paramyxoviruses. Furthermore, these observations show that RS virus also is quite distinct from rhabdoviruses and influenza viruses, virus groups that are similar to paramxyoviruses in several aspects of their molecular biology (Collins).

In earlier work (Annual report, 1984), a nucleotide sequence of the major nucleocapsid (N) protein mRNA was deduced from a cloned cDNA. Subsequent sequencing of additional, independant cDNA clones in conjunction with dideoxynucleotide sequencing of viral genomic RNA did not confirm that part of the sequence representing the 3' end of the predicted mRNA. Recently, a corrected, consensus sequence was obtained. Interestingly, the original cloned cDNA appears to represent a chimeric poly A+ transcript of a portion of the N gene and a portion of the major glycoprotein (G) gene. Such a transcript might have been generated by transcription of a defective genome containing an internal deletion (Collins).

Human RSV encodes two envelope-associated glycoproteins, the fusion (F) glycoprotein and the larger (G) glycoprotein. As described previously (Annual report, 1984), the complete amino sequence of the F glycoprotein was deduced by nucleotide sequence analysis of a nearly-complete cDNA of the mRNA. Recently, a complete cDNA of the mRNA encoding the G glycoprotein was isolated and analyzed by DNA sequencing. The predicted mRNA sequence encodes a protein of 298 amino acids, consistent with the estimated molecular weight of the <u>in vitro</u> translation product synthesized in response to hybrid-selected mRNA. Taken together with results from other laboratories, the predicted amino acid sequence shows that the G glycoprotein contains a remarkably high content of N-linked and O-linked carbohydrate (approximately 60%). The protein is highly basic, very rich in serine and threonine (33 ser and 58 thr.), and high in proline (30 pro). Most of the basic, hydroxylamino and proline residues are located in the C-terminal part of the molecule. Interestingly, hydropathic analysis identified a hydrophobic domain between amino acids 41-63 that appears to function as both a signal sequence and a membrane anchor. This suggests that the membrane orientation of the G glycoprotein resembles that of the neuramindase glycoprotein of influenza virus and the hemagglutinin-neuraminidase glycoprotein of the parainfluenza virus SV5.

Unambiguous identification of the predicted protein as the polypeptide moiety of the G glycoprotein was obtained by protein microsequencing. Radiolabeled G glycoprotein was isolated from extracts of infected cells by immunoprecipitation and gel electrophoresis and subjected to cleavage with cyanogen bromide. A large fragment was isolated by gel filtration and analyzed by amino acid microsequencing. The deduced partial sequence exactly matched that predicted from the DNA sequence, thereby providing proof of identity. Currently we are preparing the F and G cDNAs for insertion into engineered eucaryotic expression vectors. Live vaccinia virus vectors will be used to

administer the engineered genes to cotton rats (Sigmodon species) to establish the relative contribution of these viral surface antigens to the development of host immunity (Collins, Olmsted).

Human immune response to RSV infection. Infants and young children undergoing primary infection with respiratory syncytial virus (RSV) develop moderate to high levels of antibodies to the F and G surface glycoproteins of this virus, but only a very small proportion of these antibodies exhibit neutralizing activity. Possibly, epitopes on G and F glycoproteins that are not involved in virus neutralization may be immunodominant and repeated RSV infections may be required before the epitopes involved in virus neutralization can stimulate an effective immune response. The development of antibodies during primary RSV infection which are predominantly devoid of an important function required for immunity, i.e., virus neutralization, may contribute to the severity of initial infection during infancy and may also be responsible, in part, for the lack of effective resistance during early childhood to frequent reinfection by RSV and respiratory tract disease that is associated with such reinfection (Murphy, Chanock).

Experimental passive immunoprophylaxis and immunotherapy of RSV infection Parenteral inoculation of cotton rats with RSV neutralizing antibodies prior to infection reduces or prevents viral replication in the lungs. This prophylactic effect is dose-dependent and a high concentration of serum neutralizing antibodies in recipient cotton rats, i.e., greater than 1:350, is required for prevention of pulmonary infection. This suggested that parenteral administration of RSV antibodies might protect high-risk human infants from RSV infection. Sandoglobulin, a preparation of purified human IgG suitable for intravenous administration, was also highly effective in passive immunoprophylaxis in the cotton rat. Sandoglobulin was also safe and effective for therapy of RSV infection in cotton rats. When used therapeutically at the height of RSV infection, Sandoglobulin significantly decreased titer of virus in the lungs. A significant reduction in pulmonary virus titer was observed within three hours of administration of Sandoglobulin, while maximal reduction occurred after 24-48 hours. The concentration of serum antibodies in recipient animals required for a therapeutic effect was greater than that required for prophylaxis. None of the infected animals treated with Sandoglobulin developed histopathologic lesions, suggesting that Sandoglobulin therapy is unlikely to potentiate RSV disease.

A suppressive effect of Sandoglobulin therapy on serum antibody response to infection was observed. Other compartments of the immune system did not appear to be similarly affected because Sandoglobulin-treated cotton rats were immune to rechallenge with RSV, even though some of these animals lacked detectable serum neutralizing antibody at the time of rechallenge. When RSV infected, Sandoglobulin treated cotton rats were reinfected 33 to 42 days later, a normal secondary serum antibody response was observed. This suggests that the immunosuppressive effect of Sandoglobulin is limited to the infection that is treated and that a normal immune response can be anticipated during subsequent infections. Sandoglobulin was also shown to be highly effective for the prophylaxis and therapy of RSV infection of owl monkeys which represent a permissive primate model for this virus (Prince, Murphy, Chanock).

Immunologic factors in RSV disease. Twenty years ago a formalin-inactivated RSV vaccine was evaluated in young infants. The vaccine did not protect against

RSV infection despite the fact that it stimulated a moderately high serum neutralizing antibody response. When RSV infection occurred, vaccinees developed unusually severe lower respiratory tract disease. For many years we have attempted to reproduce a similar enhancement of RSV disease in laboratory animals without success. During the past year we succeeded in inducing an exaggerated RSV disease in cotton rats which have proved to be an extremely useful animal model of RSV infection.

Cotton rats inoculated previously with formalin-inactivated RSV were challenged intranasally with live RSV. Vaccinated cotton rats developed pulmonary lesions within 24 hours after infection with RSV and these lesions reached a maximum by the fourth day. Histologically the lesions resembled an experimental pulmonary Arthus reaction, although adoptive transfer experiments were not successful in confirming this mechanism. An action of formalin on RSV appears to be responsible for this effect, because live virus or virus heated in the absence of formalin did not induce enhanced immunopathology. Selected epitopes on the F and/or G RSV surface glycoproteins that are involved in inducing neutralizing antibodies are modified by formalin so as to reduce or ablate their antigenicity. However, other epitopes on the F and/or G glycoproteins are not ablated by formalin because cotton rats inoculated parenterally with formalin-inactivated virus developed a high level of F and G antibodies measurable by ELISA.

At this time the effect of formalin on RSV cannot be localized to either the F or G glycoprotein of RSV. Although the site(s) at which formalin acts to produce its disease enhancing effect has not been identified it is clear that formalin-treated RSV stimulates an unbalanced immune response in which an unusually large proportion of antibodies are directed against non-protective epitopes on RSV F and/or G. Consequently, effective resistance is not provided and the stage is set for an accelerated immune response to non-protective antigenic sites when infection occurs. Whether an accelerated immune response to non-protective epitopes contributes to enhancement remains to be determined (Prince, Murphy, Chanock).

## Human Parainfluenza Virus Type 3 (PIV3)

Molecular virology of PIV3. Human parainfluenza virus type 3 (PIV3), a paramyxovirus, is second only to RSV as a major cause of serious, acute pediatric respiratory tract disease. For this reason we initiated a program to delineate the structure and function of this virus at the molecular level. Initially we constructed and identified cDNA from PIV3 mRNAs encoding the viral hemagglutinin-neuraminidase (HN) glycoprotein, nucleocapsid phosphoprotein (P), major nucleocapsid (N) protein, matrix (M) protein, and fusion (F) glycoprotein. Northern blot hybridization identified the corresponding major viral mRNAs extracted from PI3 virus-infected cells. cDNAs of the N, P and M mRNAs were identified by hybrid-selected translation and hybrid-arrest of translation *in vitro*. A cDNA clone containing the complete HN mRNA was sequenced, and the identification of this cDNA was confirmed by comparison of the predicted protein sequence with a partial sequence obtained by direct amino acid sequencing of authenic HN protein purified from infected cells. Based on partial nucleotide sequence information for the N, P, M and F cDNAs, oligodeoxynucleotides were synthesized and used to direct dideoxynucleotide sequencing of purified viral genomic RNA. To date, this analysis has established a partial order of genes on the PIV3 genome: 3' N-P-M-(F)-HN-5'.

The sequencing of genomic RNA showed that the intergenic regions consist of the conserved nucleotide 3'-GAA-5' (genome-sense). Each gene was found to start with the conserved sequence: 3'-UCCUNNUUUC-5' (genome-sense). The identification of this sequence as the start site for gene transcription has been confirmed by dideoxynucleotide sequencing of the 5' termini of individual viral mRNAs. In contrast, the downstream termini of the PIV3 genes do not appear to conform to a conserved sequence. In this latter regard, PIV3 virus appears to differ from the prototype murine parainfluenza virus type 1 and more closely resembles measles virus. These studies will lead to a complete enumeration of the viral genes and gene products, a complete nucleotide sequence for the viral genome, and the availabilty of full-length cDNAs for engineered expression of gene products (Spriggs, Collins).

<u>Fine structure analysis of protective antigens of PIV3</u>. A parallel effort to characterize PIV3 using monoclonal antibodies yielded an operational epitope map of its hemagglutinin-neuraminidase (HN) protein. Neutralizing monoclonal antibodies (mAbs) specific for the HN protein were used to select antibody-resistant antigenic variants. Reactivity patterns of the mAbs with the antigenic variants and with isolates from pediatric patients led to the identification of 11 unique HN epitopes. Of the 11 unique epitopes defined by our antibodies, six did not undergo detectable antigenic variation in any of the 37 isolates examined. These results were expected, since human PIV3 viruses have been characterized as being antigenically monotypic. In contrast, antigenic variation was detected in the remaining five epitopes. This variation was not characterized by the accumulation of antigenic alterations with time (as occurs with for influenza A viruses), but appeared to represent genetic heterogeneity within PIV3 strains. Competitive-binding radioimmunoassays indicated that the 11 epitopes are located in two topologically distinct antigenic sites on the HN molecule (Coelingh, Murphy).

The antigenic variants achieve a maximum level of replication in cotton rat lungs and nasal turbinates which is not significantly different from that attained by wild type PIV3, indicating that the receptor-binding region of HN molecule can accomodate antigenic alterations without loss of function. Parenteral inoculation of individual mAbs that neutralize virus <u>in vitro</u> causes a 10-100 fold reduction in viral replication in the lungs of cotton rats after challenge with wild-type virus, whereas inoculation of a mixture of six mAbs reduces virus replication to an undetectable level.

Nucleotide sequence analysis of the antigenic variants is being performed to construct a molecular epitope map of the HN protein. Comparison of the HN gene sequences of three variants to that of the wild type virus identified a single point mutation in each of the variant HN genes. These mutations code for a single amino acid substitution in the HN protein which is responsible for loss of antibody-binding. Two of the variants sustained a proline to threonine substitution at amino acid position 378. The mAbs used to select these 2 variants were indistinguishable from each other in all immunologic assays and had been placed in the same epitope group in the operational epitope map of the HN protein. Sequence analysis of these variants therefore provides confirmation of our original provisional epitope map. The third variant for which the sequence analysis is complete sustained a serine to leucine substitution at amino acid position 278. The mAb used to select this variant is in a separate group from the other two in the provisional epitope map. However, all three mAbs bind competitively to the HN in radioimmunoassays,

suggesting that these epitopes, although separated by about 100 amino acids on the linear HN molecule, are brought into close proximity on the three-dimensional molecule. In addition, the three mAbs used to select these variants cross-react with the bovine strain of PIV3 but not with the human types 1, 2, or 4 parainfluenza viruses, indicating that they represent type 3-specific HN epitopes (Coelingh, Murphy).

## Molecular Biology of Dengue Viruses

Cloning the genome of dengue viruses. Dengue viruses are members of the flavivirus group of togaviridae that contain a positive strand RNA genome of approximately 12 kilobases. Many flaviviruses play an important role in human disease, for example dengue fever, yellow fever, and Japanese B encephalitis. Among the flaviviruses dengue viruses have the highest mobidity rate. With the exception of yellow fever virus vaccine, which has effectively controlled yellow fever, specific immunoprophylaxis is not generally available against diseases caused by flaviviruses. Although dengue does not constitute a major threat to public health in the U.S., dengue viruses continue to cause epidemics in many geographic areas, notably in the South Pacific, the Caribbean and Central and South America where mosquito vectors breed abundantly. In these regions dengue viruses cause large epidemics in which debilitating illness predominates. These viruses also cause a hemorrhagic shock syndrome in infants and young children; this disease has a very high mortality. The WHO has designated the dengue viruses as one of 5 high priority targets for accelerated development of virus vaccines.

Recombinant DNA techniques were employed to investigate the molecular biology of dengue virus with the intent of developing immunoprophylactic measures against this virus group. Dengue virus resembles poliovirus, another positive strand RNA virus, in that its virion RNA is infectious when inoculated into a permissive cell culture. Recombinant DNA techniques offer new opportunities to modify the genome of positive strand RNA viruses in a specific manner because cloned, full-length poliovirus DNA is infectious when introduced into a permissive cell culture. Likewise, our goal in this project is to produce a full-length, DNA copy of dengue virion RNA that is infectious in cell culture. Full length infectious dengue DNA should prove valuable for elucidating the molecular biology of the dengue viruses but more importantly it is essential for constructing mutants by site specific mutagenesis of the viral genome in its cDNA form. Manipulations of this type will be performed with the intent of producing stable, live attenuated vaccine strains.

The full-length, 42S, viral RNA of dengue virus type 4 (grown in C6/36 mosquito cells) was isolated and tailed with poly(A) at the 3'-terminus using *E. coli* poly(A) polymerase. Complementary DNA was synthesized by reverse-transcription using oligo(dT) as a primer and subsequently converted to double stranded DNA in the presence of *E. coli* RNase H, polymerase I, and ligase. The dengue cDNA products were inserted into the Pst I site of pBR322 using the dG/dC joining technique. A library of *E. coli* transformants containing dengue specific DNA inserts ranging from 2,000 - 3,500 base pairs was obtained. Thus far a total of more than 9.0 Kb of dengue DNA sequence has been identified in 5 overlapping plasmid inserts by "genome walking". A restriction enzyme map of most of the dengue DNA sequence has been constructed with these inserts. Nucleotide sequences at both termini will be determined for a number of independent clones in order to verify 3' and 5' termini. The

information that emerges from this study should allow us to use these and additional overlapping inserts to construct a full length, cloned cDNA for biologic studies (Zhao, Lai, Chanock).

The dengue virus family contains 4 distinct serotypes that are distinguishable by virus neutralization. Among the dengue group the type 2 virus is most frequently involved in hemorrhagic fever, a severe and often fatal form of dengue disease. There is considerable polymorphism among type 2 viruses as indicated by variation in oligonucleotide fingerprints and this variation has a geographic distribution in that specific patterns are limited to specific localities. Efforts have been initiated to study genetic variation of dengue viruses by molecular cloning and nucleotide sequencing. Dengue 2 (strain PR159) was chosen for sequence comparison with dengue 4 that is also being cloned and sequenced in LID. Dengue 2 genomic RNA was purified and transcribed into RNA-cDNA hybrids for direct cloning in the pBR322 vector. Analysis of Pst I digested plasmid DNA on agarose gel showed that a majority of recombinant plasmids contained DNA inserts ranging from 500-4,000 base pairs in length. Recombinants with the largest inserts (2,000 base-pairs or more) were chosen for mapping the full-length genomic sequence. For this purpose we took advantage of the genetic homology that exists between dengue virus type 2 and type 4. Radio-labelled probes prepared from cloned DNA segments of dengue 4 at various map positions were used for initial mapping and screening of dengue 2 inserts. Thus far, more than 90% of the dengue 2 genomic sequences have been cloned and our ultimate goal is to obtain a full-length DNA copy. Complete sequence analysis will be performed in an effort to gain a better understanding of the pattern of virus polymorphism in type 2 dengue epidemics and the involvement of different viral genotypes in dengue hemorrhagic fever (Lai, Zhao, Makino, Mackow, Chanock).

## Molecular Biology of Influenza A Virus

Functional analysis of signal peptide sequences of influenza A virus hemagglutinin. The influenza virus hemagglutinin (HA) is an integral membrane glycoprotein that accumulates at the surface of infected cells and is projected as a spike from the virion surface. The HA plays a key role during virus infection; it is responsible for initial attachment to the cell surface and subsequent fusion of the viral envelope with intracellular membranes. The nascent HA polypeptide contains two hydrophobic tracts of amino acid sequences: the carboxy-terminal hydrophobic region that is responsible for anchoring the protein in the cell membrane and the amino-terminal hydrophobic region that serves as a signal in the process of glycosylation, transport, and surface expression. In eukaryotic cell systems, specific interactions of the signal peptide with membranes of the endoplasmic reticulum are necessary to initiate translocation of the nascent polypeptide across the intracellular membranes.

The amino acid requirements of a functional influenza virus signal peptide were investigated using an influenza hemagglutinin (HA) cDNA-SV40 expression system in African green monkey kidney (AGMK) cells. Local, site-specific mutagenesis was carried out to generate a series of HA-SV40 recombinants containing point mutations in the region of the influenza virus hemagglutinin (HA) gene that codes for the signal peptide sequences. These mutant HA-SV40 recombinants were used to transfect AGMK cells in order to achieve expression of mutant hemagglutinins. Functional characterization of such HA products by cell surface immunofluorescence assay, hemadsorption and analysis of

glycosylation showed that a majority of the mutations, which were mostly single amino acid substitutions, had no effect on functional properties of HA. However, one isolate (mutant 28) that sustained several mutations, including an amino acid substitution at the signal cleavage site, was defective with regard to cell surface expression. Amino acid sequence analysis of the $NH_2$-terminus of mutant HA showed that the intracellularly accumulated HA failed to undergo signal cleavage. Also, the defective mutant HA contained only endoglycosidase H sensitive carbohydrate components that are added in the endoplasmic reticulum. These findings suggest that HA containing an uncleaved hydrophobic signal sequence translocates across the microsomal membrane but fails to proceed to the Golgi apparatus where endoglycosidase H resistant carbohydrates are incorporated. Point mutagenesis using a defined oligonucleotide primer has been attempted with the intention of isolating a specific cleavage mutant, ie, a mutant with an amino acid substitution only at the cleavage site. The phenotype of the mutant that is now currently being sought may allow us to confirm that signal cleavage is essential for HA translocation and cell surface expression (Lai, Markoff).

Persistent expression of cloned influenza virus genes in permissive cells. Efforts were initiated to produce persistent expression of influenza virus cloned DNA in cells permissive for virus infection. Such cells would be useful for investigation of the molecular biology of influenza virus and for isolation of specific viral mutants via complementation by the expressed gene. In this manner, naturally occurring or laboratory engineered mutants containing viable deletion mutations could be isolated for evaluation of their level of attenuation. An expression vector that contained a mutant dihydrofolate reductase (DHFR) gene as a selectable marker was employed for selection of cells expressing transfected viral DNA sequences. Alternatively, a neomycin resistance gene was used for coinfection and transformed cells were selected in the presence of G418, an analogue of neomycin. A series of recombinant DNA molecules were constructed between the DHFR expression vector and influenza nucleoprotein (NP) DNA under the control of an inducible metallothioneine promoter or a constitutive SV40 promoter. In some instances transfection of simian CV-1 cells with these DNA recombinants followed by selection with methotrexate yielded clonal cell populations; these cells were analyzed for NP synthesis. NP expressing cells were isolated at high frequency only when the inducible metallothioneine promoter was employed and a heavy metal ion inducer was added to the culture. Our failure to obtain NP expressing cells in the constitutive SV40 promoter system suggests that NP synthesis may be toxic to cell growth. Similar studies with influenza non-structural protein (NS) recombinants showed that stable synthesis of NS occurred only when the inducible system and the neomycin-resistant gene were employed. The stability of both the NP- and NS- producing cells is currently being analyzed. The expressed influenza virus protein in these cells will be analyzed for its ability to complement mutants that have a defect in that protein (Ryan, Lai, Mackow, Chanock).

Similar efforts to express the influenza PB2 polymerase protein from cloned cDNA in a permissive cell culture have been undertaken. The vector containing the mutant DHFR gene (as a selectable marker) was used in conjunction with either the constitutive SV40 promoter or the inducible metallothioneine promoter positioned just upstream from the cloned influenza PB2 cDNA. It was assumed that because PB2 binds to capped host mRNA, the constitutive expression of such a protein would be toxic to cells and therefore

cells expressing PB2 would be unstable and selected against. Inducible expression of PB2 from the metallothioneine promoter should minimize this difficulty and hence favor the establishment and stable maintainence of the PB2 gene. Simian CV-1 cells were transfected with the inducible recombinant and subjected to methotrexate selection. After 3-4 weeks, colonies of cells resistant to drug inhibition appeared. Because antiserum specific for PB2 is not available, we were unable to screen for PB2-producing cell populations by an immunoassay. However, based upon our experience with persistent expression of NP with the same vector, at least 20% of transfected cells should produce the PB2 protein. Currently, these drug-resistant cells are being analyzed for PB2 function by attempting to complement influenza mRNA transcription of PB2 mutants (Mackow, Lai, Ryan, Markoff).

## Rotaviruses

Evaluation of experimental rhesus rotavirus vaccine in infants and young children. Although the factors that mediate resistance to rotavirus disease in humans have not been defined, there is evidence from studies in experimental animals that local mucosal immunity plays a major role, suggesting that a live virus vaccine administered orally should be more effective than the parental administration of rotavirus antigens. Once the decision to pursue the development of a live rotavirus vaccine was made, we sought rotavirus strains that were satisfactorily attenuated but antigenic in susceptible individuals. Rotaviruses that met these specifications could then serve as vaccine virus strains if they possessed the protective antigen of a human rotavirus serotype. Because rotaviruses undergo gene reassortment with high efficiency during coinfection, satisfactorily attenuated rotaviruses could also serve as the donor of genes for attenuation of rotavirus reassortants bearing the protective antigens of other human rotavirus serotypes. At present, 4 serotypes of human rotavirus have been identified.

Most of our effort has been directed towards evaluating the potential usefulness of a simian (rhesus) rotavirus as a vaccine for prevention of human rotavirus disease. The rhesus rotavirus (RRV) is a promising vaccine candidate for several reasons. First, the virus appears to be restricted in humans. Thus, we were unable to detect evidence of infection of humans with this virus under natural conditions. The genes of almost all of the human rotavirus strains that have been tested hybridize under stringent conditions to labeled single-stranded (+) RNA transcripts ("probes") of a human serotype 1 or serotype 2 rotavirus indicating that these viruses belong to one of two families of human rotaviruses. However, genes of the 4 prototype human rotavirus serotypes as well as other human rotavirus isolates fail to form full-length, genomic-size hybrids when incubated with single-stranded (+) RNA transcripts prepared from RRV cores. Host restriction of RRV probably reflects the significant divergence of nucleotide sequence of its genes from that of the corresponding genes of human rotaviruses belonging to the four known serotypes. 2) The major neutralization protein (VP7) of RRV is closely related antigenically to the corresponding protein of the human serotype 3 rotavirus, which is an important cause of enteric disease during infance and early childhood. 3) RRV grows to high titer in primary simian tissue culture and has been adapted to growth in FRhL 2 cells, a semi-continuous simian diploid cell strain. The latter property may constitute a considerable advantage because adventitious agents occur with high frequency in primary monkey kidney cell cultures (Kapikian, Flores, Midthun, Chanock).

Last year safety and antigenicity of RRV vaccine was demonstrated in adult volunteers; 31 of 34 individuals developed a serologic response to RRV. These observations encouraged us to continue studies of RRV vaccine in a stepwise fashion in individuals of progressively younger age until the target population, infants less than one year of age, was reached. These studies were carried out in collaboration with scientists at 6 other institutions: Vanderbilt University (Dr. Wright); Marshall University (Drs. Belshe, Anderson); University of Maryland (Drs. Levine, Losonsky, Rennolds); University of Tampere, Tampere, Finland (Dr. Vesikari); University of Umea, Umea, Sweden (Drs. Gothefors, Waddell); National Institute of Dermatology, Caracas, Venezuela (Dr. Perez-Schael). A total of 17 separate studies were performed in which the Epidemiology Section provided major laboratory support in each instance. Following our initial studies in young children who had pre-existing serum RRV antibody, it was necessary to revise our plan to administer RRV vaccine to seronegative children 2 - 12 years of age because almost all children in this age group possessed RRV antibody. For this reason we then studied individuals 6 months - 2 years of age who had serum RRV antibody. Subsequently, seronegative individuals in the this age group were evaluated.

Initial analysis of studies in the United States revealed that the RRV vaccine was quite antigenic; 49 (86%) of 57 infants and young children 4 months to 12 years of age (including 19 [83%] of 23 who were 4-12 months of age) developed serologic evidence of infection following feeding of RRV. In addition, shedding of RRV was detected in 77% of the vaccinees for up to 10 days post-inoculation. Virus was detected predominantly by tissue culture methods since most stools were negative when tested by conventional ELISA. This indicated that RRV was moderately restricted in susceptible individuals. In addition, significant reactions such as fever, diarrhea and vomiting were not associated with the RRV vaccine when it was fed at a dose of $10^7$ pfu (undiluted) or $10^6$ pfu (1:10 dilution) to 64 infants and young children 4 months - 12 years of age, including 26 who were 4-12 months of age. Although vaccine and placebo recipients at the University of Maryland had the same frequency of fever, vomiting or diarrhea, there was clustering of fevers on days 3 and 4 in the vaccine group (Kapikian, Flores, Midthun, Glass, Chanock).

Because significant reactions to RRV vaccine were not observed, further studies were initiated in infants 6 months - 1 year of age. In a phase I study at the University of Tampere, Finland, (December 1984) 49 infants 6-8 months of age received either RRV ($10^6$ pfu) or Smith-Kline RIT-4237 vaccine (bovine rotavirus). Sixteen (64%) of 25 RRV recipients and only 3 (12.5%) of 24 RIT-4237 recipients developed a transient fever indicating that RRV induced transient febrile reactions in 6 - 8 month old infants, whereas the RIT vaccine was less reactogenic. In contrast, the RRV vaccine was demonstrably more antigenic than the bovine rotavirus vaccine; 76% fed the former vaccine had a seroresponse, while the latter vaccine induced a seroresponse in only 52% of recipients (Kapikian, Glass). A study of RRV vaccine in infants in Umea, Sweden confirmed the findings made during the Finnish trial (Glass, Kapikian).

At this point we sought a dose of vaccine which would cause few if any reactions in the 4-12 month age group. The effect of dose on reactivity of the RRV vaccine was evaluated in a phase I study in 4-10 month old infants in Caracas, Venezuela. Seventeen infants were fed $10^5$ pfu of vaccine, 18 were fed $10^4$ pfu and 19 received a placebo. There was no significant difference in the occurrence of fever (≧100.6°F), diarrhea or vomiting in the 3 groups. In

addition, 82% of the vaccinees who received $10^5$pfu and 59% who received $10^4$pfu developed a seroresponse when tested by a variety of assays. Failure to detect reactions in this population may be related to a higher level of prevaccination serum RRV antibody than in the preceding trials. Most prevaccination antibody appeared to be the result of prior natural infection, while passive transfer of maternal antibody could have been the source in several instances. Approximately one half of the vaccinees who received $10^5$pfu of vaccine shed RRV, while 30% who received $10^4$pfu of vaccine shed rotavirus (Flores, Kapikian).

Although the RRV vaccine offers several advantages as a potential vaccine strain, such as growth to relatively high titer in FRhL 2 cells, antigenic similarity to human serotype 3 rotavirus, and high infectivity and immunogenicity in humans, it clearly induced transient febrile reactions in infants in Finland and Sweden. The absence of febrile reactions in the U.S. during studies at Vanderbilt and Marshall Universities can be attributed to pre-existing immunity as indicated by the significantly higher prevaccination serum RRV antibody titer of U.S. vaccinees, 4-12 months, compared to Finnish infants. The absence of reactions in the Venezuela study is encouraging but probably also reflects pre-existing immunity as indicated by a high level of prevaccination serum RRV antibody.

In developing countries the impact of rotavirus diarrhea is greatest during early infancy when this virus group accounts for 50% of serious acute enteric disease. Hence, it will be necessary to administer rotavirus vaccine during the first 1-2 months of life. This is a time when the titer of passively acquired serum RRV antibodies is quite high. Also live oral poliovirus vaccine, which may interfere with RRV, is routinely administered during this period. It should be noted that neonates exhibit a unique response to rotavirus in that most infections are asymptomatic. Whether resistance to disease in early life is due to passively acquired maternal antibody or is a manifestation of host physiologic factors unique to the neonatal period is not clear. In any case, RRV is sufficiently infectious for man that it may infect most neonates, despite their inherent resistance, and initiate a silent immunizing infection. The RRV vaccine strain has a 1000 fold range over which it can infect susceptible humans. Within this range it may be possible to identify a dose of RRV that can initiate a silent immunizing infection in partially resistant neonates. Whether the desired balance between attenuation and immunogenicity can be achieved will be the subject of clinical studies now being planned for next year. Unlike RRV, the RIT bovine rotavirus vaccine has a very narrow range of infectivity for infants and the vaccine quickly loses its infectivity for highly susceptible individuals upon dilution. Also the infectivity of the RIT vaccine for young infants is inhibited by simultaneous feeding of live oral poliovirus vaccine. This suggests that a rotavirus vaccine strain with greater infectivity will be required for successful immunization of very young infants who are fed poliovirus vaccine simultaneously. Possibly, the more infectious RRV strain may prove satisfactory in these circumstances. The results of clinical trials planned for next year should decide this issue. Finally, there is reason to be optimistic concerning the effectiveness of early immunization against rotaviruses. Observations made during a recent longitudinal study by Dr. Bishop (Melbourne, Australia) indicate that neonatal rotavirus infection (during the first 14 days of life) induces resistance to rotavirus disease and this protective effect lasts for at least 3 years.

Rotavirus genetics. Reassortant viruses with characteristics that make them potential vaccine candidates have been isolated from coinfection of primary simian tissue culture with a fastidious human rotavirus (strains D, DS-1, P, or ST3, representing serotypes 1, 2, 3, or 4, respectively) and a wild type bovine or rhesus rotavirus. Analysis of the genotypes of these reassortants revealed that many derived 10 genes from the animal rotavirus parent and only one gene, that which codes for the major neutralization protein, $VP_7$, from the human rotavirus parent. These reassortants represent promising candidate live vaccine strains because their animal rotavirus gene complement should produce attenuation while the major neutralization protein of human rotavirus should induce protective immunity. These single human rotavirus gene substitution reassortants have been adapted to growth in DBS-FRhL cells (Midthun, Hoshino).

Extensive analysis of these and other reassortants by neutralization indicates that a gene product other than $VP_7$ is also involved in neutralization. This other gene product appears to be the outer capsid protein VP3, coded for by the 4th gene. Attempts are being made to isolate reassortants which derive both their VP3 and VP7 genes from the human rotavirus parent and the remaining 9 genes from the animal rotavirus parent (Hoshino, Midthun).

During the course of characterizing a variety of rotaviruses of mammalian and avian origin by the plaque reduction neutralization technique, several rotaviruses that bore neutralization antigens of two distinct serotypes were identified. During the past year, efforts were made to define the basis for this "intertypic bridging" phenomenon. In several instances it was shown that the presence of closely related VP3 antigens on viruses that possessed very disparate VP7 antigens was responsible for "intertypic bridging". Thus, in addition to VP7, the VP3 protein contains antigenic sites which stimulate and react with neutralizing antibodies and the sharing of VP3 neutralization sites by two viruses results in cross neutralization. These observations indicate the need to establish a new system for antigenic characterization of rotaviruses in which both the VP3 and VP7 neutralization proteins are identified. Similar observations concerning the role of VP3 antigens in virus neutralization were made during the characterization of certain reassortant viruses described above (Hoshino, Midthun, Flores, Kapikian).

Rotavirus single stranded (ss) RNAs prepared by in vitro transcription from viral cores and cloned rotavirus cDNA were used as probes in hybridization reactions to investigate the molecular epidemiology of human rotaviruses and to assess the extent of genetic diversity among human and animal rotavirus strains. Homology was shown to be greatest among rotavirus strains isolated from the same animal species. Also it was observed that genetic variation in human rotaviruses was rather common. Rotaviruses undergo genetic variation by two mechanisms: a) accumulation of successive mutations within the genome (genetic drift); and b) gene reassortment (genetic shift) that results in the appearance of rotavirus strains with a new constellation of genes which are derived from two or more distinct rotaviruses. The relative importance of these two mechanisms (genetic drift or shift) in the generation of new strains is not clear at this time. Partial sequence analysis of nosocomial rotavirus strains recovered from neonates who underwent asymptomatic infection in a hospital nursery over a one year interval suggests that the rotavirus genome does not have a high rate of spontaneous mutation. However, on occasion novel rotavirus strains can emerge through gene reassortment. Evidence that gene

reassortment occurs under natural conditions was provided by the identification of several human rotaviruses that exhibited human rotavirus serotype specificity by neutralization but failed to hybridize to a ss RNA probe of Wa (serotype 1) or DS-1 (serotype 2) rotavirus. Almost all human rotaviruses hybridize to either the Wa or DS-1 probe (Flores, Midthun, Hoshino).

Sequence relatedness among individual rotavirus genes was also studied by Northern blot analysis. In these studies individual gene segments were initially separated by polyacrylamide gel electrophoresis (PAGE) and then blotted onto DBM paper. Labeled ss RNA transcripts or rotavirus cDNA probes were then hybridized to the blotted RNAs under different conditions of stringency. This procedure permitted us to estimate the extent of homology of individual genes of the strains being analyzed to the corresponding genes of the strain used to prepare the probe. Viruses representative of each of the 4 human serotypes were examined. These virus strains were recovered from ill children as well as from asymptomatic newborn infants. Several animal rotaviruses were also examined. Under conditions of low stringency (which allows up to 25% mismatch) all the corresponding genes from the different strains exhibited homology. At high stringency (less than 12% mismatch allowed), the relationships were more specific. The fifth gene (in order of electrophoretic migration), which encodes a non-structural protein of unknown function, was most divergent. The fourth gene appeared to be highly conserved among the human rotavirus strains recovered from ill children, irrespective of serotype. Similarly, the fourth gene was conserved among the strains which were isolated from asymptomatic newborn infants, irrespective of serotype. However, the fourth gene of the newborn strains was not closely related to the fourth gene of strains isolated from sick children. Perhaps, the fourth gene of the newborn rotavirus strains is responsible for the diminished virulence exhibited by these viruses (Flores, Midthun, Hoshino, Kapikian).

The VP7 glycoprotein genes of strains belonging to different serotypes can sometimes be distinguished under conditions of high stringency if these genes can be easily resolved from other genes that migrate nearby during gel electrophoresis. Under conditions that permitted resolution of the VP7 gene from its near neighbors, VP7 cross-hybridization was observed only among strains within the same serotype. The remaining genes, ie, 1, 2, (or 2-3), 6, 7, 8, 10 and 11, seem to be conserved among the rotavirus strains studied, irrespective of their origin (human or animal) (Flores, Midthun, Hoshino, Kapikian).

<u>Rotaviruses recovered from asymptomatic infection of human neonates</u>. Nineteen rotavirus strains derived from asymptomatic neonates (seven from England, five from Australia, two from Venezuela, and five from Sweden) were successfully cultivated in primary African green monkey kidney cell culture. Serotype was determined by plaque reduction neutralization (PRN) assay. Each of the 19 strains belonged to one of the four known human serotypes; serotype 1 (both Venezuelan strains), serotype 2 (all Swedish strains), serotype 3 (all Australian strains), or serotype 4 (all English strains). Hyperimmune guinea pig antiserum raised against the Venezuelan strain (M37) neutralized the prototype serotype 1 (Wa) virus and the prototype serotype 4 (St. Thomas no. 3) virus to a similar degree. The intertypic reactivity of the M37 rotavirus resulted from a sharing of VP7 (the major neutralization protein) with serotype 1 (Wa) and a sharing of VP3 (the other neutralization protein) with serotype 4 (St. Thomas). These observations confirm that VP3 possesses antigenic sites

that induce and react with neutralizing antibodies (Hoshino, Midthun, Kapikian).

Molecular biology of rotaviruses. cDNA clones were constructed for diverse genes of various rotavirus strains. These include the human strains Wa, DS1, Price, M-37 and ST3 and animal strains NCDV, RRV, UK and OSU. Transformation of E.coli with rotavirus-pBR322 recombinant plasmids yielded a large number of clones carrying rotavirus gene copies. The rotavirus cDNA present in each clone was identified by Northern blot hybridization or colony hybridization with cDNAs of known gene origin. Clones that contained cDNA of the VP3 gene (that encodes the outer capsid hemagglutinin) from the simian RRV and the bovine UK rotavirus have been identified and the VP3 gene of RRV has been partially sequenced. In addition, clones containing cDNA of the VP7 gene of several animal rotaviruses (OSU, NCDV, RRV) and several human rotavirus strains have been identified and characterized by restriction mapping. Several of the VP7 clones have been sequenced partially or in their entirety (Flores, Glass, Gorziglia). For example, the full sequence of the bovine NCDV VP7 glycoprotein gene was determined as well as most of the sequence of the porcine OSU and simian RRV VP7 genes. These rotaviruses represent different serotypes and their VP7 sequences are now being compared to each other and to previously published sequences in order to identify regions of sequence divergence that may code for major antigenic sites involved in virus neutralization. Comparison of the deduced amino acid sequence of NCDV (serotype 6) VP7 to that of four other rotavirus strains (human Wa serotype 1, human HU-5 serotype 2, simian SA-11 serotype 3, and bovine UK serotype 6) indicated that the degree of amino acid homology among VP7 neutralization proteins of these serotypes ranged from 75 to 86%. Four hydrophilic regions bounded by amino acid residues 174-183, 248-256, 287-294, and 310-317 exhibited significant homology and hence may represent common antigenic determinants, while one hydrophilic area bounded by amino acid residues 83-102 exhibited sufficient divergence to suggest that it may be involved in serotype specificity (Glass, Flores).

The cloned sequence of the NCDV VP7 gene is 1062 nucleotides in length. The 5' end of VP7 exhibits the greatest homology among diverse rotavirus strains; the first 10 nucleotides are completely conserved and there are few differences in the next 62 base pairs. At the 3' end, the last 15 nucleotides are conserved in all strains. The first potential initiation codon at nucleotides 49-51 begins the longest reading frame while a second potential initiation codon in the same reading frame is present at nucleotides 136-138. A TAG codon at residues 1027-1029 terminates the open reading frame of each of the rotavirus VP7 genes sequenced thus far (Glass, Flores). A full size cDNA copy of the VP7 gene of porcine rotavirus OSU has also been cloned and its restriction pattern analyzed. We are in the process of sequencing this gene and transferring it to bacterial expression vectors such as the vector system developed by Dr. Inouye (Stoneybrook University) (Gorziglia, Flores).

A complete cDNA copy of the VP3 gene (approximately 2400 base pairs) has not been recovered thus far but partial cDNA copies of the Wa, RRV and UK VP3 genes have been identified. A VP3 gene clone of RRV representing ±1500 base pairs has been partially sequenced. It represents the 5' end of the VP3 ss RNA (ie, mRNA). Attempts to obtain clones representing the 3' end are are in progress. VP3 is of some importance because it undergoes post-translational cleavage and this event is required for virus infectivity. Also VP3 appears to be a major determinant of host range restriction as well as a target for a

subset of antibodies that neutralize virus infectivity (Flores, Gorziglia, Glass).

Expression of bovine rotavirus VP7 by vaccinia-VP7 recombinant. In collaboration with Dr. Moss (LVD), a vaccinia virus recombinant which expresses bovine rotavirus VP7 (the major neutralization protein) was constructed by inserting a cDNA copy of the complete VP7 gene of NCDV into the TK gene of vaccinia virus. This recombinant virus expressed a polypeptide of approximately 35,000 daltons which migrated closely with VP7 of NCDV. Two rabbits vaccinated intradermally with this recombinant virus developed a significant increase in serum NCDV VP7 antibodies as measured by indirect immunofluorescence. Rabbits, hamsters and mice possessed neutralizing antibodies directed against the VP7 of NCDV prior to vaccination with the recombinant and this made it difficult to interpret the post-vaccination neutralizing antibody response of these animals to NCDV (O. Nakagomi, Flores).

Attempts to express rotavirus VP7 in E.coli. Partial digests of the VP7 gene of bovine rotavirus or RRV were inserted in several different inducible prokaryotic vectors and evidence of expression was sought. A high level of expression was achieved in several instances but the fusion protein produced was not recognized by monoclonal or polyclonal antisera (T. Nakagomi, O. Nakagomi, Flores).

Genomic rearrangement during serial passage in cell culture. An unusual rearrangement of the bovine NCDV genome was observed following initial high multiplicity infection of MA104 cells followed by 14 serial passages in these cells. The 5th gene segment of plaque purified virus from the 14th tissue culture passage could not be detected by gel electrophoresis of viral RNAs. Instead a new RNA segment was detected that migrated between RNA segments 1 and 2. RNA-RNA hybridization analysis indicated that there was homology between the new RNA segment and the 5th gene of parental virus. The mechanism of this rearrangement is not understood and clearly merits additional study (Askaa).

## Norwalk-like 27nm Viruses as Etiologic Agents in Acute, Non-bacterial, Gastroenteritis.

The Marin County agent is a 27nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents as assessed by immune electron microscopy (IEM) or solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers were later fed a 20ml inoculum. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Examination by IEM of several diarrheal stool specimens from this volunteer demonstrated a large number of 27nm particles. These particles were shown to be identical to the Marin County agent in IEM studies using acute and convalescent sera from the original outbreak. A preliminary survey of a series of gastroenteritis

outbreaks using a recently developed RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis (Midthun, Kapikian).

## Honors and Awards

### Robert Chanock

Elected Class Membership Committee, National Academy of Sciences.

Member of Council of American Society of Virology.

Co-organizer and co-chairman of Third Cold Spring Harbor Conference on Modern Approaches to Vaccines, Cold Spring Harbor, NY, September 11-15, 1985.

Appointed to Advisory Council of Scripps Research Foundation, La Jolla, Calif.

Invited participant, Banbury Center, Cold Spring Harbor Laboratory Conference on Genetically Altered Viruses on the Environment, Cold Spring Harbor, NY, April 28 - May 1, 1985.

Steering Committee of WHO, Program For Vaccine Development, Acute Respiratory Viruses, Geneva, Switzerland, July 29-30, 1985.

Associate editor of Virology textbook, Raven Press, New York, 1985.

### Albert Kapikian

Invited to make presentation on "Recent Advances in studies of etiology of human viral gastroenteritis" at Conference on "New Approaches to Control of Viral Infections", June 4-5, 1984; sponsored by the American Institute in Taiwan and Coordination Council for North American Affairs at Fogarty International Center, NIH, Bethesda, Md. Presentation on June 4, 1984.

Invited to attend and participate in a meeting of the World Health Organization Steering Committee of the Scientific Working Group on Viral Diarrheas in Tokyo, Japan. Dr. Kapikian is a member of the steering committee. Aug 28-30, 1984.

Invited to make a presentation on "Development of Rotavirus Vaccines" at Sixth International Congress of Virology, Sendai, Japan, September 1-7, 1984. Presentation on September 2, 1984.

Invited to attend Working Conference on Rabies, Arboviruses, including Dengue and Viral Gastroenteritis sponsored by Japan-U.S. Cooperative Medical Science Program, September 9-11, 1984 in Oiso, Japan. Dr. Kapikian made a presentation on "Studies in volunteers with rhesus rotavirus strain MMU 18006 as a candidate vaccine for humans" on September 9, 1984. He was

also co-chairman with Dr. Konno of the session on Viral Gastroenteritis on September 9, 1984.

Invited to make presentation at Cold Spring Harbor Symposium on "Modern Approaches to Vaccines", September 12-16, 1984 Presentation on September 16, 1984 on "Rhesus rotavirus strain MMU 18006: a candidate vaccine for humans". (Attended September 13-16, 1984).

Invited to be co-chairman (with Dr. Blacklow) at Session on "Rotavirus Infections" on October 9, 1984 at 24th Interscience Conference on Antimicrobial Agents and Chemotherapy (sponsored by American Society for Microbiology) in Washington, D.C. (Meeting dates: October 8-10, 1984)

Invited to be co-moderator of session (with Cedric Mims) at meeting on "Viral and Mycoplasmal Infections of Laboratory Rodents: Effects on Biomedical Research, October 24-26, 1984. Session 2 on Basic Biology and Pathogenic Mechanisms on October 24, 1984.

Invited to be on membership committee of American Epidemiological Society, 1985.

Invited to attend and participate in Eighth Meeting of the Steering Committee of the Scientific Working Group on Viral Diarrheas of the World Health Organization, Jan 30-31, 1985, Geneva, Switzerland. (Member of Steering Committee)

Invited to make presentation at Seminar on "Viral Vaccines" at Annual Meeting of the American Society for Microbiology, Las Vegas, Nevada (March 3-7, 1985) Made presentation on "Rotavirus" on March 4, 1985. (Attended March 4-7.)

Invited to be co-convenor of seminar on March 6, 1985 on "Recent Advances in the etiology and prevention of viral gastroenteritis" at the Annual Meeting of The American Society for Microbiology Meeting, Las Vegas, Nevada. (March 3-7, 1985.)

Invited to make presentation on "Alternative approaches to the development of vaccines against viral diarrheal disease" at Nobel Conference on "Recent Advances in Vaccines and Drugs Against Diarrheal Diseases" in Saltsjobaden, Stockholm, Sweden. June 3-6, 1985. Presentation on June 5, 1985.

Invited to be chairman of a session at Nobel Conference in "Recent Advances in Vaccines and Drugs Against Diarrhoeal Diseases" in Saltsjobaden, Stockholm, Sweden. June 3-6, 1985. Chaired closing session on June 6, 1985.

Robert H. Purcell

Recipient, Inventor's Incentive Award, Commerce Department, 1984.

Invited participant, Five-year Review of Hepatitis Panel, U.S.-Japan Bilateral Science Agreement, Tokyo, Japan, July 16-19, 1984.

Invited participant and Session Chairman, Sixth International Congress of Virology, Sendai, Japan, September 1-7, 1984.

Recipient, Gold Medal awarded by Canadian Liver Foundation, Montreal, Canada, September 11, 1984.

Invited speaker, Annual Meeting of the Canadian Society for Clinical Investigation, Canadian Association of Gastroenterology and the Royal College of Physicians of Canada, September, 1984.

Invited speaker, Symposium on Modern Approaches to Vaccines, Cold Spring Harbor, New York, September 12-16, 1984.

Invited speaker, Annual Meeting, The Royal College of Pathologists of Australasia, Perth, Australia, October 15-19, 1984.

Invited speaker, 1984 Annual Forbes Oration, Fairfield Hospital, Melbourne, Australia, October 23, 1984.

Invited speaker, National Cancer Institute Workshop on AIDS, Bethesda, Maryland, December 10-11, 1984.

Invited participant, Annual Meeting U.S.-Japan Bilateral Science Agreement, Viral Hepatitis Panel, Oiso, Japan, March 11-12, 1985.

Invited Consultant, Review of National Hepatitis Control Program, Taipai, Formosa, March 14-15, 1985.

Invited participant, Ad Hoc Committee Meeting on Viral Hepatitis, PAHO, Rio de Janeiro, May 13-17, 1985.

Invited participant, Meeting of Scientific Session of Steering Committee of the WHO Hepatitis A Vaccine Development Programme, Bergen, Norway, May 21, 1985.

Invited participant, 9th Scandinavian Virus Symposium, Bergen, Norway, May 20-22, 1985.

Invited participant, WHO Steering Committee on Hepatitis, Geneva, Italy, July 1-2, 1985.

Brian Murphy

Public Health Service Meritorious Service Award, 1985.

Keynote speaker, Eastern Chapter of American Society of Microbiology, Philadelphia, PA., February 1985.

Yasutaka Hoshino

Invited to make presentation of the 18th Working Conference on Rabies,

Arboviruses including Dengue and Viral Gastroenteritis sponsored by the Viral Diseases Panels, U.S.-Japan Cooperative Medical Sciences Program, Oiso, Japan, September, 1984. Made presentation on "Role of the fourth gene in cross-neutralizing reactions among various rotaviruses".

Invited to make presentation of the 6th International Congress of Virology, Sendai, Japan, September, 1984. Made presentation on "Serotypic characterization of rotaviruses derived from asymptomatic human neonatal infections".

Karen Midthun

Invited to present at the Working Conference on Rabies, Arboviruses Including Dengue and Viral Gastroenteritis sponsored by Japan-U.S. Cooperative Medical Science Program, September 9-11, 1984, in Oiso, Japan. Title of presentation: "Construction of reassortant rotaviruses for vaccine development".

Served as Laboratory of Infectious Diseases Representative on the National Institute of Allergy and Infectious Diseases Intramural Program Safety Committee, NIH, Bethesda, MD., 1984-1986.

Roger Glass

Awarded Ph.D. from the University of Goteborg, Sweden, 1984.

Jorge Flores

Member of the National Commission on Biotechnology, Venezuela, 1984-1985.

Rebecca Tominack

Awarded Certification by the National Board of Internal Medicine, 1985.

Ching-Juh Lai

Presented NIH Director Award, June 1985.

Invited to make presentation at the Annual Biochemistry Society Meeting of Germany, GBCH, Giessen, West Germany, Sept 17- 20, 1984. Title of presentation: "Functional analysis of influenza viral antigens synthesized from cloned DNA sequences.

Invited discussant to the International Workshop on the Molecular Biology of Flaviviruses, Fort Detrick, MD., Nov 29-Dec 1, 1984.

Lewis Markoff

Received qualification for Board Certification in Clinical Infectious Diseases, The Johns Hopkins Hospital and Medical School, Baltimore, MD., 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00308-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vitro Studies of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS (if any)

Smith-Kline-RIT (Dr. d'Hondt); Office of Biologics, FDA (Dr. Daemer)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Hepatitis Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.1 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. Over 28 serial passages have been achieved, with infectivity titers as high as $10^8$ infectious units per ml of cell concentrate. The virus is predominantly cell-associated and does not produce cytopathic effects (CPE). It was attenuated for chimpanzees after 10 serial tissue culture passages; reevaluation of the virus in chimpanzees after 20 tissue culture passages indicates the virus infectivity and attenuation have remained the same as at passage level 10. The attenuated virus has been shown not to revert to virulence during serial passage through chimpanzees. Immunization of chimpanzees with the attenuated virus is protective. The tissue culture-adapted HAV has been 3X cloned. These clones have been characterized in chimpanzees and marmosets.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00309-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Biology of Hepatitis A Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |
| | John Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| | Bahige Baroudy, Ph.D. | Visiting Associate | LID, NIAID |

COOPERATING UNITS (if any) Fairfield Hospital, Melbourne, Australia (Dr. I. Gust); Office of Biologics, FDA (Dr. Daemer)

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 1.3 | 0.7 | 0.6 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have successfully isolated a strain of hepatitis A virus in African green monkey kidney tissue culture, a cell substrate suitable for vaccine development. Growth of the agent *in vitro* has been characterized and attenuation for chimpanzees documented. The strain of virus appears to have considerable potential for vaccine development.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00310-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Hybridoma Antibodies to Pathogenic Viruses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| Others: | Jingsing Mao, M.D. | Visiting Fellow | LID, NIAID |
| | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |

COOPERATING UNITS (*if any*) NIAID, NIH, Bethesda, Maryland (Dr. A. Fauci)

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Production and characterization of monoclonal antibodies to hepatitis A virus are in progress. The development of new serologic tests for detection of monoclonal anti-HAV has simplified the procedure. Five specific monoclonal antibodies have been produced and are being analyzed.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00311-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Search for New Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: | Albert Kapikian, M.D. | Head, Epidemiology Sect. | LID, NIAID |
| | John Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |

COOPERATING UNITS *(if any)* Clin. Center Blood Bank, NIH, Bethesda, MD (Dr. Alter); Nat. Inst. Virol., Pune, India (Dr. Pavri); Med. College Srinagar, India (Dr. Khuroo); Mt. Sinai Hosp., NYC (Dr. Popper); Inst. Pasteur d'Algerie, Algiers, (Dr. Belsbbes)

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.9 | 0.5 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by recognized hepatitis viruses. Attempts to transmit an agent from acute-phase clinical samples to primates are in progress. Attempts to transmit an agent from clinical specimens from these outbreaks have been partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies have been observed in some animals, but this has not been uniform, and attempts to serially transmit an agent in chimpanzees and marmosets have also produced irregular results. Characteristic histopathologic changes distinct from those seen in type A hepatitis, type B hepatitis and non-A non-B hepatitis have been reported by Dr. Hans Popper (Mt. Sinai, New York).



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00312-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical and Experimental Studies of Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John Ticehurst, M.D. | Senior Staff Fellow | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |

COOPERATING UNITS *(if any)* Baylor School Med., Houston, TX (Dr.Hollinger); Div. Mol. Virol. & Immunol., G.U., Wash. DC (Dr. Gerin); Dept. Med., Rutgers Med. School, New Brunswick, NJ (Dr. McAuliffe); CDC, Atlanta, GA (Dr. Francis); Dept. Epid., Shanghai 1st Med. Coll. (Dr. Xu).

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.3 | 0.2 | 0.1 |

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical testing and characterization of plasma-derived hepatitis B vaccine prepared by the NIAID have been completed. The vaccine is highly immunogenic, safe and well tolerated when tested in healthy persons ranging from infants to adults. Results from an efficacy trial in Asia suggest that the vaccine effectively prevents transmission of hepatitis B virus infection from mothers to infants.

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00313-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Hepatocellular Carcinoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: | Kendo Kiyosawa, M.D. | Visiting Associate | LID, NIAID |
| | He Li-Fang, M.D. | Visiting Fellow | LID, NIAID |

COOPERATING UNITS *(if any)*

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|---|---|---|
| 0.8 | 0.7 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing the tumor. These antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential, cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we sought immunofluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contained an antibody that reacted with a nuclear antigen in the hepatoma cell line. This antigen was found in another hepatoma cell line that also contained integrated HBV genome but not in two other hepatoma cell lines lacking HBV genome. The antigen ("hepatitis B virus-associated nuclear antigen": HBNA) is being further characterized to determine if it is the product of a transforming gene. A different nuclear antigen was found in a human hepatoma cell line that did not contain HBV DNA. It was identified with serum from a patient with HBsAg negative hepatocellular carcinoma. The new antigen has characteristics similar to those of HBNA.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00314-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Woodchuck Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Robert Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (if any) Div. Molec. Virol. & Immunol., Georgetown U, Washington, DC (Dr. Gerin); New York State College of Veterinary Medicine (Dr. B. Tennant)

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|---|---|---|
| 0.2 | 0.1 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus (HBV). Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. They are being applied to a prospective study of WHV infection in newborn woodchucks and the ability of active immunization to prevent hepatic cell carcinoma. The results of the study should have important prognostic value in evaluating active immunoprophylaxis of hepatitis B virus in man. Preliminary observations indicate that WHV vaccine protects chimpanzees against HBV infection, however, HBV vaccine does not protect woodchucks against WHV infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00315-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Search for New Hepadnaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | John Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |

COOPERATING UNITS *(if any)* Veterinary Resources Branch, NIH (R. Whitney); Div. Molecular Virology, Georgetown U., Washington, D.C. (P. Coté)

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|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Viruses similar to hepatitis B virus ("hepadnaviruses") have been identified in three non-human species: the eastern woodchuck, the Beechey ground squirrel and the Pekin duck. It is likely that many other species harbor similar viruses. The existing animal hosts are of limited value because inbred strains suitable for detailed immunological studies are not available. An attempt was made to transmit woodchuck hepatitis virus to cotton rats and guinea pigs but we could not detect evidence of infection. We are also searching for hepadnaviruses among inbred strains of rodents, especially those with a known high incidence of hepatoma, in hopes of finding a more useful animal model system. Sensitive assays of hepadnavirus infection have been modified to permit testing of the small quantities of serum available.

T E R M I N A T E D



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00316-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Delta Agent

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS *(if any)* Georgetown U., Washington, DC (Dr. Gerin); CDC, Phoenix, Arizona (Dr. Hadler); Sinai Hospital, NYC (Dr. Popper); Chiba U., Chiba City, Japan (Dr. Omata); U. Washington Med. Research Unit, Taipei, Taiwan (Dr. Beasley)

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|---|---|---|
| 0.4 | 0.1 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The delta agent is a transmissible hepatitis agent that appears to be defective and requires co-infection with hepatitis B virus for its own synthesis. The agent has a small RNA genome ($10^{5.7}$ daltons) that is encapsidated together with delta antigen within a coat of HBs Ag. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a hepatitis virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important agent. Recently, serologic studies of Yucpa Indians (Venezuela) indicated that delta agent was etiologically associated with severe and often fatal hepatitis in this population which has a high incidence of hepatitis B virus infection. Serologic evidence that the delta agent is associated with severe hepatitis in Brazil (Labrea fever) and Colombia (Santa Marta hepatitis) has also been obtained.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00317-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biology of Non-A, Non-B Hepatitis Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| | He Li Fang, M.D. | Visiting Fellow | LID, NIAID |

COOPERATING UNITS (if any) Blood Bank, NIH Clinical Center, Bethesda, MD (Dr. Alter, Dr. Shih, Dr. Shiraishi); CH, LMG, NIH, Bethesda, MD (Dr. Dawid, Dr. Sargent).

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|---|---|---|
| 1.0 | 0.8 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titers of various plasmas that contain non-A, non-B virus. Although most plasmas contain only $10^2$-$10^3$ infectious units per ml, one plasma was found to contain over $10^6$ infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least one non-A, non-B agent contains essential lipids, a characteristic that will be important in the classification and, probably, the control of non-A, non-B hepatitis.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00318-03 LID |
|---|---|

PERIOD COVERED
October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Non-A, Non-B Hepatitis Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |
| Others: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS *(if any)*

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|---|---|---|

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attempts to identify non-A, non-B hepatitis agents by serologic means have been uniformly unsuccessful throughout the world. We have sought to apply recent advances in nucleic acid chemistry to a search for the genome of the non-A, non-B agent. Sensitive radiolabeling procedures have been modified to permit labeling of minute quantities of nucleic acid. Identification of the genome of the non-A, non-B agent would permit its characterization and cloning.

TERMINATED



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00319-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

New Approaches to the In Vitro Propagation of Non-Cultivatable Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS *(if any)*

Office of Biologics, FDA (Dr. Daemer)

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.1 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is a need for an in vitro substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely in vitro. Methods for the selection of hybrid cells without drug markers (i.e. use of vital dyes) have been developed.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00321-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Development, Characterization, and Use of Cloned Hepatitis A Virus cDNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*


| | | | |
|---|---|---|---|
| PI: | John Ticehurst, M.D. | Senior Staff Fellow | LID, NIAID |
| Others: | Stephen M. Feinstone, M.D. | Medical Officer | LID, NIAID |
| | Robert H. Purcell, M.D. | Head, HV Sect. | LID, NIAID |
| | Bahige M. Baroudy, Ph.D | Visiting Associate | LID, NIAID |
| | Nickolaos Tassopoulos, M.D. | Visiting Scientist | LID, NIAID |
| | Jeffrey I. Cohen, M.D. | Medical Staff Fellow | LID, NIAID |
| | Manfred Weitz, Ph.D. | Fogarty Visiting Fellow | LID, NIAID |


COOPERATING UNITS *(if any)* Col. U., NY (Racaniello); MIT, Cmbrdg MA (Baltimore); Chiron Corp., CA (Dina); U. Cal., Brkly (Tracy); U. Wisc. (Palmenberg); U. Leicester, UK (Almond); U. Turku, Finland (Hyypia); Genentech Corp., CA (Kleid); USDA, Plum Is. NY (Moore); Scripps Clinic (Lerner); UNC (Sobsey); LMB, NCI (Maizel); LIG, NIAID (Maloy).

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 0.8 | 1.7 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Double-stranded cDNA fragments were synthesized from hepatitis A virus (HAV) RNA and inserted into the Pst I site of pBR322. The identity of cloned cDNA was established by demonstrating its hydridization to RNA from HAV-infected tissue culture cells but not to RNA from uninfected cells. Genomic length RNA of approximately 7500 nucleotides was the predominant species that hybridized with the cloned HAV cDNA. Cloned cDNA from near the 5' terminus of the genome was used to synthesize and clone cDNA by primer extension so that a molecular clone was obtained that contained the 5' terminus of the genome. Restriction endonuclease digestion and hybridization between subgenomic fragments yielded a map of overlapping cloned cDNAs of the complete viral genome. Cloned cDNA was used as a probe for detecting HAV RNA in tissue culture, serum, and fecal specimens by hybridization. Hybridization experiments also demonstrated that probes taken from any region of the HAV genome will not hybridize to RNA or cloned cDNA from a variety of other picornaviruses, a result that was supported by comparison of nucleotide sequences using computer programs. Ligation between overlapping cloned cDNAs resulted in a complete representation of the HAV genome in pBR322 and attempts are being made to produce virus from this clone by transfection.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00370-03 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Studies of Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (*if any*) NINCDS, NIH, Bethesda, MD (Dr. London); NCI, NIH (Dr. Gallo); New York Blood Center, NYC, NY (Dr. Stevens, Dr. Baker); Memorial Sloan Kettering Inst., NYC, NY (Dr. Gold); LIG, NIAID, NIH, Bethesda, MD (Dr. Folks, Dr. Fauci); Georgetown U. (Dr. Gerin).

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.2 | 0.1 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

A new medical syndrome, Acquired Immune Deficiency Syndrome (AIDS) has recently been recognized. It is characterized by profound progressive depression of the immune system, resulting in repeated opportunistic infections and at least one type of neoplasm.

The syndrome is usually if not always fatal. It is epidemic in the United States among certain "high-risk populations (male homosexuals, illicit drug users, Haitians, and, to a lesser extent, hemophiliacs, recipients of blood transfusions and intimate contacts of cases).

A new retrovirus has been identified as the etiologic agent of AIDS. Successful transmission of the virus to chimpanzees has been reported. Attempts to find a more suitable primate animal model are in progress.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00404-02 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Second and Third-Generation Hepatitis B Vaccines

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS (*if any*) Georgetown University, Washington, DC (Dr. Gerin); Scripps Institute, La Jolla, California (Dr. Lerner); LBV, NIAID, NIH, Bethesda, MD (Dr. B. Moss)

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| 0.1 | 0.1 | 0 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Expense, availability and/or incomplete acceptance based upon an unfounded fear of infection with the agents of AIDS limit the impact of plasma-derived hepatitis B vaccines in developed and developing countries. There is therefore a need for new approaches to vaccine development. Recombinant DNA and synthetic peptide technologies appear to offer the best opportunities for the next generation of hepatitis B vaccines. Partial or complete protection against hepatitis B has been demonstrated in chimpanzees following vaccination with (a) recombinant derived subunit vaccine prepared in eukaryotic cells, (b) live recombinant vaccinia virus containing HBV genes, and (c) synthetic peptides representing HBsAg sequences. Attempts to identify the antigenic domains most important in stimulating neutralizing antibody are currently in progress.



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PROJECT NUMBER

Z01 AI 00405-02 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inactivation of Hepatitis Viruses in Pooled Plasma Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert H. Purcell, M.D. Head, HV Section LID, NIAID

COOPERATING UNITS *(if any)* Georgetown U., Washington DC (Dr. Gerin); Southwest Fndt., San Antonio, TX (Dr. Eichberg); Revlon Inc., Tuckahoe, NY (Dr. Landaburu)

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| 0.2 | 0.1 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The transmission of viral hepatitis by plasma derivitives continues to be an important medical problem that has been made more urgent by the similar epidemiology of the putative agent of acquired immune deficiency syndrome. Methods of inactivating viruses in plasma derivatives while retaining the biological potency of the products are being explored. These include the application of heat to lyophilized biological products and extraction of lyophilized or aqueous preparations with lipid solvents such as chloroform.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00406-02 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Structure of HAV: Genomic Sequence and Organization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


| | | | |
|---|---|---|---|
| PI: | Bahige M. Baroudy, Ph.D. | Visiting Associate | LID, NIAID |
| Others: | Robert H. Purcell, M.D. | Head, HB Section | LID, NIAID |
| | John R. Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| | Stephen Feinstone, M.D. | Medical Officer | LID, NIAID |
| | Manfred Weitz, Ph.D. | Visiting Fellow | LID, NIAID |


COOPERATING UNITS (if eny) Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, NIH, Bethesda, MD (Dr. Jacob V. Maizel). Laboratory of Immunogenetics, NIAID, NIH, Bethesda, MD. (Dr. L. Walter Maloy).

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|---|---|---|
| 1.4 | 1.0 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


We have sequenced several HAV cDNA clones by the Maxam and Gilbert technique and to date to two large regions of the genome have been determined. A sequence of 3119 bases corresponding to the 5' end of HAV RNA was sequenced. This sequence contains an open reading frame that begins ≅750 bases from the 5' terminus and extends 2407 bases which is as far as sequence has been determined. Sequence preceding this major reading frame has nine other potential initiation sites, but the longest peptide that can be translated in this region is only 26 amino acids. This pattern is consistent with the genomic organization of other picornaviruses. The entire sequence of a clone that mapped to the 3' end of HAV RNA was also determined. A poly(A) tract of 15 bases was found at one end of this clone thus orienting the 3' end of the genome. This poly (A) tract is 51 bases downstream from two closely spaced termination codons that are preceded by 1407 bases in an open reading frame that is presumably continuous with that present at the 5' end of the genome. These sequenced regions of the HAV genome have been compared, using computer programs, to analogous regions previously determined for other picornaviruses. In the past year we have sequenced sufficient clones to span 7.0kb of the HAV genome. Morever, we were able to deduce from this data the putative sequence of VPg, a protein believed to be covalently attached to the 5' end of the genome. In collaboration with Dr. Maloy, we synthesized a peptide corresponding to the carboxy end of this protein and were able to obtain antisera that immunoprecipitated the VPg-RNA genome complex.




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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00440-01 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Construction of Full Length Hepatitis A Virus cDNA for Transfection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jeffrey I. Cohen, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | John R. Ticehurst, M.D. | Medical Staff Fellow | LID, NIAID |
| | Stephen M. Feinstone, M.D. | Medical Staff Fellow | LID, NIAID |
| | Robert H. Purcell, M.D. | Head, HV Section | LID, NIAID |

COOPERATING UNITS *(if any)* Columbia University, NY (Dr. Racaniello)

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|---|---|---|
| 0.8 | 0.8 | 0 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNA from hepatitis A virus (HAV) has been cloned into pBR 322. Six cDNA clones which together span the entire genome of HAV have been isolated. These clones have been ligated together to form a single clone which was thought to represent a cDNA analog of full length HAV in pBR 322. In addition, the HAV cDNA was inserted into an SV40 vector. Transfection of both tissue culture cells (*in vitro*) and marmosets (*in vivo*) with these plasmids failed to generate HAV. Fine structure mapping of the HAV cDNA subsequently indicated that about 40 base pairs had been deleted during the ligation process. Construction of a full length infectious HAV cDNA clone is in progress.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00322-03 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Amino Acid Sequence of RS Viral Nucleocapsid (N) Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*


PI: Peter L. Collins, Ph.D. Senior Staff Fellow LID, NIAID

Others: Sundararajan Venkatesan, M.D. Expert LID, NIAID
(present address, Frederick Cancer Research Center)
Narayanasamy Elango, Ph.D. Visiting Associate LID, NIAID
(present address, LVD, NIAID)


COOPERATING UNITS *(if any)*

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


In earlier work (N. Elango and S. Venkatesan, 1983, Nucleic Acids Research, 11: 5941-5551; annual report, 1984), a nucleotide sequence of the major nucleocapsid (N) protein mRNA was deduced from a cloned cDNA. Subsequent sequencing of additional, independant cDNA clones in conjunction with dideoxynucleotide sequencing of viral genomic RNA could not confirm part of the sequence representing the 3' end of the predicted mRNA. Here, a corrected, consensus sequence obtained from these studies is described. Interestingly, the original cloned cDNA appears to represent a chimeric poly A+ transcript of portions of the N and major glycoprotein (G) genes. Such a transcript might have been generated by transcription of a defective genome containing an internal deletion.


TERMINATED



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00323-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure of Parainfluenza Type 3 Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Peter L. Collins, Ph.D. | Senior Staff Fellow | LID, NIAID |
| Others: | Melanie K. Spriggs, Ph.D. | Staff Fellow | LID, NIAID |
| | Narayanasomy Elango, Ph.D. | Visiting Associate | LID, NIAID |
| | S. Venkatesan, M.D. | Expert | LID, NIAID |
| | Robert C. Jambou | Biologist | LID, NIAID |
| | Kathleen Coelingh, Ph.D. | Senior Staff Fellow | LID, NIAID |
| | Robert A. Olmsted, Ph.D. | Staff Fellow | LID, NIAID |
| | Alicia Buckler-White, Ph.D. | Staff Fellow | LID, NIAID |

COOPERATING UNITS (if any)

LIG, NIAID (Dr. John E. Coligan)

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|---|---|---|
| 2.2 | 1.7 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have constructed and identified cDNA classes of human parainfluenza virus type 3 (PI3 virus) mRNAs encoding the viral hemagglutinin-neuraminidase (HN) glycoprotein, nucleocapsid phosphoprotein (P), major nucleocapsid (N) protein, matrix (M) protein, and fusion (F) glycoprotein. Northern blot hybridization identified the corresponding major viral mRNAs extracted from PI3 virus-infected cells. cDNAs of the N, P and M mRNAs were identified by hybrid-selected translation and hybrid-arrest of translation *in vitro*. A cDNA clone containing the complete HN mRNA was sequenced, and the identification of this cDNA was confirmed by comparison of the predicted protein sequence with a partial sequence obtained by direct amino acid sequencing of authenic HN protein purified from infected cells. Based on partial nucleotide sequence information for the N, P, M and F cDNAs, oligodeoxynucleotides were synthesized and used to direct dideoxynucleotide sequencing of purified viral genomic RNA. To date, this analysis determined a partial order of genes on the PI3 genome: 3' N-P-M-(F)-HN-5'. Further sequence analysis of cDNAs and viral genomic RNA will provide a complete enumeration of viral genes and a complete determination of viral gene sequences.



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PROJECT NUMBER

Z01 AI 00324-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Laboratory Studies of Myxoviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*


| | | | |
|---|---|---|---|
| PI: | Mark H. Snyder, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Alicia Buckler-White, Ph.D. | Staff Fellow | LID, NIAID |
| | Brian R. Murphy, M.D. | Head, RV Section | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |


COOPERATING UNITS *(if any)* U. of Michigan (Dr. Maassab, Dr. DeBorde)

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|---|---|---|
| 3.15 | 1.15 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Avian-human influenza A reassortant viruses containing human influenza hemagglutinin and neuraminidase genes and the six internal genes of either the avian influenza A/Mallard/78 (H2N2) or A/Pintail/79 (H4N6) virus are attenuated for monkeys and humans. The NP and M genes of the avian influenza A/Mallard/78 virus are individually capable of producing attenuation. NP genes of three avian influenza A viruses were found to have significant divergence of their amino acid sequences from those of three human influenza A viruses. Unlike the M gene of avian influenza A/Mallard/78 virus, the M gene of avian influenza A/Pintail/79 virus is incapable by itself of attenuating an avian-human influenza A reassortant virus. A specific constellation of polymerase genes of the avian influenza A/Pintail/79 virus produces a host range specific restriction of viral replication in MDCK tissue culture. Studies with single gene reassortant viruses derived from the human influenza A/Ann Arbor/60 ca donor virus show that the NP and NS genes of the ca virus are individually not important in producing the attenuated ca phenotype. Single gene M, NP, and NS reassortants were not ts.




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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00325-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Respiratory Viruses in Primates

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Brian R. Murphy, M.D. | Head, RV Sect. | LID, NIAID |
| Others: | Mark H. Snyder, M.D. | Medical Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |

COOPERATING UNITS (if any) Meloy Laboratories (Dr. Jere Philips), Rockville, MD; NINCDS, NIH (Dr. William T. London); WRAIR, Division of Areobiology (Dr. Edward Stevenson) Frederick, MD

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|---|---|---|
| 1.0 | 0.7 | 0.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The newly constructed "six gene" avian-human influenza A reassortant viruses derived by mating the avian influenza A/Pintail/79 or A/Mallard/76 virus with the human influenza A/Korea/82 wild type virus were shown to be satisfactorily attenuated and immunogenic in monkeys prior to evaluation of these reassortants in man.

The RNA 1 and NS genes, but not the M gene, of the A/Pintail/79 virus contribute to the host range restriction of this virus in monkeys. In this regard the genetic basis for host range restriction of the avian influenza A/Pintail/79 virus reassortants differs from that of the previously studied avian influenza A/Mallard/78 virus reassortants in which the avian influenza M gene plays a major role in attenuation. Because different genes in different avian influenza A viruses appear to be responsible for host range restriction it may be possible to construct a "composite" avian influenza donor virus with 4 or more attenuating "internal" genes (derived from different avian influenza A viruses) in order to ensure genetic stability of reassortant vaccine viruses.

The "six gene" influenza A _ca_ reassortant virus derived from the influenza A/Ann Arbor/6/60 _ca_ donor virus and the human influenza A/Korea/82 wild type virus, which replicates efficiently _in vitro_ at 37°C, was greatly restricted in replication in the lower respiratory tract (37°C) of the chimpanzee. This indicates that the A/Ann Arbor/6/60 _ca_ donor virus has sustained host range mutations during passage in chick kidney cells. Similarly, two different avian-human influenza A reassortant viruses were greatly restricted in the lower respiratory tract of the chimpanzee, confirming the restriction of replication of these reassortants in a non-human primate closely related to man.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00326-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Study of Respiratory Viruses in Volunteers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Brian R. Murphy, M.D. | Head, RV Sect. | LID NIAID |
| Others: | Mark H. Snyder, M.D. | Medical Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |

COOPERATING UNITS (if any) Flow Labs. (Potash), Rockville, MD; U. of Md. Sch. Med. (Clements) Balt., MD; U. Rochester Sch. Med. (Betts, Dolin), Roch., NY; Vanderbilt U. Sch. of Med. (Wright), Nashville, TN; Marshall U. Sch. of Med. (Belshe), Huntington, W.Va.; FDA (Burlington), Bethesda, MD.;

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|---|---|---|
| 2.55 | 0.55 | 2.0 |

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- [x] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The six "internal" genes of the avian influenza A/Mallard/6750/78 virus reproducibly attenuated three human influenza A viruses belonging to two different antigenic subtypes. Infection with one of the avian-human influenza A reassortant viruses induced resistance to challenge with homologous wild type virus. Two avian-human influenza A reassortant viruses derived from the avian influenza A/Pintail/79 virus were overattenuated for volunteers.

The PA polymerase gene of the influenza A/Ann Arbor/6/60 cold-adapted (ca) donor virus appears to play a major role in the attenuation of human influenza A viruses. A "six-gene" ca reassortant virus can infect and induce protective levels of immunity in over half of seropositive and about 90% of seronegative volunteers.

The ca reassortant vaccines induce resistance to homologous wild type virus challenge that persists seven months post-vaccination at a level slightly greater than that induced by inactivated vaccine. However, ca reassortant immunity does wane by seven months as indicated by a partial loss of protection against infection and upper respiratory tract illness. This decrease in resistance correlates with a three-fold decrease in the level of nasal wash IgA antibody. The mediators of immunity induced by inactivated vaccine are serum HAI antibody, serum NI antibody, and nasal wash IgG HA antibody. The mediators of immunity induced by infection with ca vaccines are serum NI, and nasal wash IgA HA antibody.




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PROJECT NUMBER

Z01 AI 00327-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Parainfluenza Type 3 and RS Viruses with Monoclones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Kathleen L. van Wyke Coelingh, Ph.D. | Senior Staff Fel. | LID, NIAID |
| Others: | Peter L. Collins, Ph.D. | Senior Staff Fel. | LID, NIAID |
| | Gregory Prince, D.D.S., Ph.D. | Expert | LID, NIAID |
| | Brian R. Murphy, M.D. | Head, RV Sect. | LID, NIAID |
| | Judy A. Beeler, M.D. | IPA | LID, NIAID |

COOPERATING UNITS (if any) Battelle Memorial Institute, Cleveland, OH (Dr. J. Rice); Baylor College of Medicine, Houston, TX (Dr. J. Kasel).

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|---|---|---|
| 2.7 | 1.7 | 1.0 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


We have constructed an operational epitope map of the type 3 parainfluenza virus (PIV3) hemagglutinin-neuraminidase (HN) protein. Neutralizing monoclonal antibodies (mAbs) specific for the HN protein were used to select antibody-resistant antigenic variants. Reactivity patterns of the mAbs with the antigenic variants and with human clinical strains have identified 11 unique HN epitopes. Five of these epitopes vary antigenically in clinical strains whereas variation in six other epitopes is extremely rare. Competitive-binding radioimmunoassays indicated that the 11 epitopes are located in two topologically distinct antigenic sites on the HN molecule.

The antigenic variants achieve maximum levels of replication in cotton rat lungs and nasal turbinates which are not significantly different from that achieved by wild type PIV3, indicating that the HN molecule can accomodate antigenic alterations without loss of function. Passive transfer of individual mAbs that neutralize virus <u>in vitro</u> causes a 10-100 fold reduction in viral replication in cotton rat lungs after challenge with wild-type virus, whereas transfer of a mixture of six mAbs reduces virus replication to an undetectable level.

Nucleotide sequence analysis of the antigenic variants is being performed to construct a molecular epitope map of the HN protein. Comparison of the HN gene sequences of three variants to that of the wild type virus identified a single point mutation in each of the variant HN genes. These mutations code for a single amino acid substitution in the HN protein which is responsible for loss of antibody-binding.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00344-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunologic Factors in Respiratory Syncytial Virus (RSV) Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Gregory A. Prince, D.D.S., Ph.D. | Expert | LID, NIAID |
| Others: | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |
| | Brian R. Murphy, M.D. | Head, RVS, LID | LID, NIAID |

COOPERATING UNITS *(if eny)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.95 | 0.45 | 0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Cotton rats previously inoculated with formalin-inactivated RSV were challenged intranasally with live RSV in an attempt to experimentally induce an enhancement of RSV disease similar to that observed following administration of formalin-inactivated RSV vaccine to infants 20 years ago. Within 24 hours after infection with RSV cotton rats developed pulmonary lesions that reached a maximum by the fourth day. Histologically the lesions resembled an experimental pulmonary Arthus reaction, although adoptive transfer experiments were not successful in confirming this mechanism. An action of formalin on RSV appears to be responsible for this effect, because live virus or virus heated in the absence of formalin did not induce enhanced immunopathology. Selected epitopes on the F and/or G RSV surface glycoproteins that are involved in inducing neutralizing antibodies were modified so as to reduce or ablate their antigenicity. However, other epitopes on the F and/or G glycoproteins are not ablated by formalin because cotton rats inoculated parenterally with formalin-inactivated virus developed a high level of F and G antibodies measurable by ELISA.

At this time the effect of formalin on RSV cannot be localized to either the F or G glycoprotein of RSV. Although the site(s) at which formalin acts to produce its disease enhancing effect has not been identified it is clear formalin-treated RSV stimulates an unbalanced immune response in which an unusually large proportion of antibodies are directed against non-protective epitopes on RSV F and/or G. Consequently, effective resistance is not provided and the stage is set for an accelerated immune response to non-protective antigenic sites when infection occurs. Whether an accelerated immune response to non-protective epitopes plays a major role in enhancement remains to be determined.





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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00345-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunity to RS Virus

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Gregory Prince, D.D.S., Ph.D. | Expert | LID, NIAID |
| Others: | Robert M. Chanock, M.D. | Chief | LID, NIAID |
| | Brian R. Murphy, M.D. | Head, RVS | LID, NIAID |

COOPERATING UNITS (*if any*)

USUHS, Bethesda, MD (Dr. Hemming); Childrens Hospital National Medical Center, Wash., D.C. (Dr. Rodriguez).

LAB/BRANCH

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Respiratory Viruses Section

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.95 | 0.45 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the spece provided.*)


Parenteral inoculation of cotton rats with RSV neutralizing antibodies prior to infection reduces or prevents viral replication in the lungs. This prophylactic effect is dose-dependent and a high concentration of cotton rat serum neutralizing antibodies, i.e., greater than 1:350, is required for prevention of pulmonary infection. This suggested that parenteral administration of RSV antibodies might protect high-risk human infants from RSV infection. Sandoglobulin, a preparation of purified human IgG suitable for intravenous administration, was also highly effective in passive immunoprophylaxis in the cotton rat. Sandoglobulin was also safe and effective for therapy of RSV infection in cotton rats. When used therapeutically at the height of RSV infection, Sandoglobulin significantly decreased titer of virus in the lungs. A significant reduction in pulmonary virus titer was observed within three hours of administration of Sandoglobulin, while maximal reduction occurred after 24-48 hours. None of the infected animals treated with Sandoglobulin developed histopathologic lesions, suggesting that Sandoglobulin therapy is unlikely to potentiate RSV disease.

A suppressive effect of Sandoglobulin therapy on serum antibody response to infection was observed. Other compartments of the immune system did not appear to be similarly affected because Sandoglobulin-treated cotton rats were immune to rechallenge with RSV, even though some of these animals lacked detectable serum neutralizing antibody at the time of rechallenge. When RSV infected, Sandoglobulin treated cotton rats were reinfected 33 to 42 days later, a normal secondary serum antibody response was observed. This suggests that the immunosuppressive effect of Sandoglobulin is limited to the infection that is treated and that a normal immune response can be anticipated during subsequent infections. Sandoglobulin has also been shown to be highly effective prophylactically and therapeutically in owl monkeys which constitute a permissive primate model for RSV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00367-02 LID

PERIOD COVERED
October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
RS Viral Nonstructural Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Sundararjan Venkatesan, M.D. | Expert | LID, NIAID |
| Others: | N. Elango, Ph.D. | Visiting Associate | LID, NIAID |
| | M. Satake, Ph.D., M.D. | Visiting Associate | LID, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies indicate that two RS viral nonstructural proteins (NS1 and NS2) are coded for by two adjacent genes. By means of positive hybrid selection of viral mRNAs and subsequent in vitro translation of the selected RNAs a recombinant RS viral plasmid (pRSC$_6$) that encoded two viral nonstructural proteins was identified. This plasmid that contained a RS viral insert of about 1,050 bases hybridized to viral mRNAs containing about 500-600 bases as indicated by Northern blot analysis. The insert was sequenced completely and found to contain two different nonoverlapping reading frames that coded for proteins that contained 139 and 124 amino acids respectively. Upstream of the second reading frame there was a nine nucleotide GGGGCAAAT sequence between positions 545-553. The recombinant pRSC$_6$ was thus shown to be a cDNA clone of a bicistronic transcript. Bicistronic transcript(s) of RS virus may originate because of a failure of the viral polymerase to pause at the intergenic region.

T E R M I N A T E D



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01-AI-00368-03 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structural Analylsis of Respiratory Syncytial Virus Genome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Peter L. Collins, Ph.D. | Senior Staff Fellow | LID, NIAID |
| Others: | Robert A. Olmsted, Ph.D. | Staff Fellow | LID, NIAID |
| | Melanie K. Spriggs, Ph.D. | Staff Fellow | LID, NIAID |
| | Alicia Buckler-White, Ph.D. | Staff Fellow | LID, NIAID |
| | Kathleen Coelingh, Ph.D. | Senior Staff Fellow | LID, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human respiratory syncytial (RS) virus, an enveloped virus that contains a single negative-sense strand of genomic RNA, is an important agent of pediatric respiratory tract disease. Previously, complete cDNAs and complete nucleotide sequences were obtained for nine of the ten known viral mRNAs. Here, synthetic oligodeoxynucleotides were used to direct dideoxynucleotide sequencing of intergenic and flanking regions in the viral genome. The results confirmed the sequences of the gene termini obtained from the cDNAs, showing that the nine viral mRNAs, initiate with the conserved sequence 5' GGGGCAAAUA$^{A}_{U}$.. and terminate with the conserved sequence 5' ... AGU$^{A}_{U}$ A(N)$_{1-4}$ - poly A. Comparison of the intergenic and flanking sequences with the complete mRNA sequences established unambiguously the 3' to 5' order of the nine genes on the viral genome. Each gene was immediately followed (in genome-sense) by an oligo U tract of 4-7 residues that might direct synthesis of poly A tails of the mRNAs by a reiterative copying mechanism. The intergenic regions varied in length from 1 to 52 nucleotides and displayed no obvious sequence conservation except that in all cases the last nucleotide (in genome-sense) was an A residue.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00371-02 LID |
|---|---|

PERIOD COVERED
October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Nucleotide Sequence of the Genes Encoding RS Viral M and P Proteins

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute effiliation)*

| | | | |
|---|---|---|---|
| PI: | Sundararajan Venkatesan, M.D. | Expert | LID, NIAID |
| Others: | Masanobu Satake, M.D., Ph.D. | Visiting Associate | LID, NIAID |
| | N. Elango, Ph.D. | Visiting Associate | LID, NIAID |

COOPERATING UNITS (*if any*)

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Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| | | |

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


We determined the amino acid sequence of RS viral M protein was deduced from the cDNA sequence of a recombinant plasmid harboring the gene. The RS viral cDNA insert of 950 nucleotides had a poly(A) tail at one end. The other end corresponding to the 5' end of the mRNA lacked five nucleotides (NGGGC) of the mRNA. The cDNA sequence had an open reading frame capable of encoding a protein of 28717 dal (256 amino acids). The protein was relatively basic and moderately hydrophobic. It did not contain regions homologous to other viral matrix proteins. A second open reading frame potentially encoding a protein of 75 amino acids was also present at the 3' end of the cDNA insert. This overlapped the first reading frame by 20 amino acids. Several recombinant plasmids containing cDNA encoding the RS viral phosphoprotein gene were identified by a variety of methods. $pRSA_3$ encoding RS viral P protein was selected for sequencing. It has 916 bp of RS viral sequence including a poly A tail of 14 residues. It lacked the NGGG...sequence corresponding to the 5' end of the mRNA. As with other RS viral genes this is part of the conserved sequence 5' NGGGCAAAT. Starting at position 18, there is a single long open reading frame encoding a protein of 241 amino acids with a molecular weight of 27150. It lacks sequence homology with Sendai virus P protein or VSV NS protein which represent counterparts of RS P protein. Unlike the situation reported for Sendai and measles viruses, this gene does not have a second reading frame capable of encoding another protein.


T E R M I N A T E D

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00372-03 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Respiratory Syncytial Virus Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*


| | | | |
|---|---|---|---|
| PI: | Peter L. Collins, Ph.D | Senior Staff Fellow | LID,NIAID |
| Others: | Masanobu Satake, M.D., Ph.D. (Present address: Litton Industries) | Visiting Associate | LID, NIAID |
| | Narayanasamy Elango, Ph.D. (Present address: LVD, NIAID) | Visiting Associate | LID, NIAID |


COOPERATING UNITS *(if any)*

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Laboratory of Infectious Diseases

SECTION

Respiratory Viruses Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.3 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Human respiratory syncytial (RS) virus encodes two envelope-associated glycoproteins, the fusion (F) glycoprotein and the larger (G) glycoprotein. As described previously (annual report, 1984; Elango et al., 1985), the complete amino sequence of the F glycoprotein was deduced by nucleotide sequence analysis of a nearly-complete cDNA of the mRNA. Recently, a complete cDNA of the mRNA encoding the G glycoprotein was isolated and analyzed by DNA sequencing. The predicted mRNA sequence encodes a protein of 298 amino acids, consistent with the estimated molecular weight of the *in vitro* translation product synthesized in response to hybrid-selected mRNA. Taken together with results from other laboratories, the predicted amino acid sequence shows that the G glycoprotein contains a remarkably high content of N-linked and O-linked carbohydrate and probably is anchored in the membrane by a hydrophobic domain located near the N-terminus.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00457-01 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Immune Response to Respiratory Syncytial Virus Infection or Vaccination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Brian R. Murphy, M.D. | Head, RV Sect. | LID, NIAID |
| Others: | Gregory Prince, D.D.S. | Expert | LID, NIAID |
| | Judy A. Beeler, M.D. | IPA | LID, NIAID |
| | Kathleen Coelingh, Ph.D. | Senior Staff Fel. | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, | LID, NIAID |

COOPERATING UNITS *(if any)* U. of Rochester Sch. Med.(Walsh), Roch., N.Y.; Childrens Hospital Nat. Med. Center (Kim), Wash., D.C.; NCI (Nelson, Wagner), NIH Bethesda, MD., Vanderbilt U. (Wright), Nashville, TN.

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Respiratory Viruses Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.7 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infants and young children undergoing primary infection with respiratory syncytial virus (RSV) develop moderate to high levels of antibodies to the F and G surface glycoproteins of this virus, but only a very small proportion of these antibodies exhibit neutralizing activity. The development of antibodies during primary RSV infection which are predominantly devoid of an important function required for immunity, i.e., virus neutralization, may contribute to the severity of initial infection and may also be responsible, in part, for the ineffectiveness of resistance to frequent subsequent reinfection by RSV, with associated respiratory tract disease, during early childhood.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00329-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Expression of the Influenza A Virus Neuraminidase Glycoprotein from Cloned DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |
| Others: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
National Institutes of Health, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.5 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A full-length ds DNA copy of the virion RNA segment coding for an influenza A neuraminidase (NA) glycoprotein was previously cloned into the late (deleted) region of an SV40 shuttle vector. The influenza-specific product of a lytic infection with this vector was shown to be glycosylated and inserted in the outer cell membrane. Additional studies established that weak enzymatic activity of the vector-coded NA was detectable in lysates of infected cells. Three deletion mutant NA DNAs that lacked sequences coding for 7 (dlK), 21 (dlI) or all 23 amino acids (dlZ) of the N-terminal hydrophobic region in the wild-type NA were studied in similar fashion, and a comparison of the phenotypes of these mutants suggested that this region functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation. Experiments are now in progress to induce point mutations in DNA coding for the hydrophobic N-terminus of the NA protein to determine whether alterations in this region may result in: (1) a membrane anchorage defect which would result in secretion of the mutant polypeptide, (2) altered processing as indicated by a change of glycosylation pattern, or (3) altered transport.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00331-03 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcription of Influenza A Virus: Synthesis of Spliced and Unspliced mRNAs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |

COOPERATING UNITS *(if any)* Dept. of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois (Dr. R. A. Lamb)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Influenza virus RNA segment 8 codes for two distinct proteins, $NS_1$ and $NS_2$, that are translated from separate mRNA's. Mapping and sequencing studies have shown that the $NS_1$ mRNA is a colinear transcript and $NS_2$ mRNA contains a spliced region. In order to investigate the splicing potential of influenza virus mRNA derived from virion RNA segment 8, cloned full-length NS DNA was inserted into the late region of an SV40 expression vector and the recombinant used for infection of primate cells. Sizing by nuclease $S_1$ analysis and nucleotide sequencing indicated that both interrupted and uninterrupted mRNA's containing influenza NS sequences were synthesized in cells infected with the recombinant. The sequences found at the junction of the interrupted mRNA were identical to those found in the $NS_2$ mRNA produced in influenza virus infected cells. These studies establish that during influenza virus infection processing of the NS mRNA transcript involves a mechanism of splicing similar to that which occurs with DNA-directed RNA transcription. Our observations thus eliminate other possible explanations for interrupted mRNAs such as "transcription from defective interfering particles and transcriptional jumping."

TERMINATED



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00332-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985 — INACTIVE

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Engineering the Genome of Influenza Virus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Lewis J. Markoff, M.D. | Medical Officer | LID, NIAID |
| | Kevin Ryan, Ph.D. | Staff Fellow | LID, NIAID |
| | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |

COOPERATING UNITS (if any) The Wistar Inst., Philadelphia, Pa. (Jonathan Yewdell)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our goal has been to use recombinant DNA techniques to construct influenza virus mutants with deletions in strategic regions of the genome. Viable deletion mutants would be especially valuable for use in immunoprophylaxis since these mutants would be unlikely to revert and therefore should be stable as regards phenotype. With this goal in mind, we produced full-length cloned DNA sequences of gene segments of an H3N2 influenza A virus. Thus far we have cloned and characterized 6 full-length genes (hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), matrix protein (M), non-structural proteins (NS), and polymerase protein PB2): the remaining two genes (PB1 and PA) have also been cloned but not in complete form. These full-length DNA clones should produce corresponding RNA transcripts that contain the control sequences needed for transcription and replication of viral genes. The validity of this prediction was established for transcription and expression of viral proteins. Functional influenza viral protein (HA, NA, or NP) was produced when simian cells were transfected with a SV40 recombinant vector containing cloned influenza DNA. The influenza cDNA was inserted into the late region of SV40 in an orientation which resulted in transcription of (+) strand influenza RNA. Attempts to rescue cloned influenza DNA by coinfection of transfected cells with influenza A virus were unsuccessful.

INACTIVE



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00365-03 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Signal Sequences of Influenza Virus Hemagglutinin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |
| | Alicia Buckler-White, Ph.D. | Staff Fellow | LID, NIAID |
| | Brian R. Murphy, M.D. | Head, RV Section | LID, NIAID |

COOPERATING UNITS (if any)

Laboratory of Immunogenics, NIAID, NIH, Bethesda, MD. (Dr. John Coligan)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Molecular Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.6 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The amino acid requirements of a functional influenza virus signal peptide were investigated using the influenza hemagglutinin (HA) cDNA-SV40 expression system in African green monkey kidney (AGMK) cells. Local site-specific mutagenesis was carried out to generate a series of recombinants of HA-SV40 containing point mutations in the region of the influenza virus hemagglutinin (HA) gene that codes for the signal peptide sequences. These mutant HA-SV40 recombinants were used to transfect AGMK cells in order to achieve expression of mutant hemagglutinins. Functional characterization of such HA products by cell surface immunofluorescence assay, hemadsorption and analysis of glycosylation showed that a majority of the mutations had no effect on functional properties of HA. However, one isolate (mutant 28) that sustained several mutations including an amino acid substitution at the signal cleavage site was defective with regard to cell surface expression. Amino acid sequence analysis of the $NH_2$-terminus of mutant HA showed that the intracellularly accumulated HA failed to undergo signal cleavage. Also, the defective mutant HA contained only endoglycosidase H sensitive carbohydrate components that are added in the endoplasmic reticulum. These findings suggest that HA containing an uncleaved hydrophobic signal sequence translocates across the microsomal membrane but fails to proceed to the Golgi apparatus where endoglycosidase H resistant carbohydrates are incorporated. Point mutagenesis using a defined oligonucleotide primer has been attempted with the intention of isolating a specific cleavage mutant that will allow us to confirm that the signal cleavage defect present in mutant 28 is indeed responsible for its defect in HA translocation and cell surface expression.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00366-03 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biology of Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| Co-PI: | Banghti Zhao, Ph.D. | Visiting Associate | LID, NIAID |
| | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Robert M. Chanock | Chief, LID | LID, NIAID |

COOPERATING UNITS *(if any)*

Dept. Virus Diseases, Walter Reed Army Inst. of Research, Washington, D.C.
(Dr. Walter Brandt)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.3 | 1.2 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue viruses are members of the flavivirus group of togaviridae that contain a positive strand RNA genome of approximately 12 kilobases. We employed recombinant DNA techniques to investigate the molecular biology of dengue virus with the intent of developing immunoprophylactic measures against this virus group that is epidemic in many geographical areas. The 42S full-length RNA from dengue virus type 4, produced in C6/36 mosquito cells, was isolated and tailed with poly(A) at the 3'-terminus using E. coli poly(A) polymerase. Complementary DNA was synthesized by reverse-transcription using oligo(dT) as a primer and subsequently converted to double stranded DNA in the presence of E. coli RNase H, polymerase I, and ligase. The dengue cDNA products were inserted into the Pst I site of pBR322 using the dG/dC joining technique. A library of E. coli transformants containing dengue specific DNA inserts ranging from 2,000 - 3,500 base pairs was obtained. From these inserts a restriction enzyme map of an almost full-length dengue DNA sequence has been constructed by "genome walking". Nucleotide sequences at both termini will be determined and verified to facilitate the construction of a full-length cloned DNA for further biologic studies.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00369-03 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Persistent Expression of Influenza Virus Polymerase Proteins from Cloned DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| Others: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| | Kevin Ryan, Ph.D. | Staff Fellow | LID, NIAID |
| | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION
Molecular Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
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| 1.0 | 0.8 | 0.2 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the spece provided.)

We investigated the functional activity of the influenza polymerase PB2 protein and initiated attempts to establish persistent expression of PB2 polymerase functions by transfecting cloned PB2 DNA into permissive cells. Functional expression of the polymerase genes should provide a complementation system for growth of influenza virus mutants with mutations affecting these genes. This type of growth complementation should be useful for isolating naturally occurring or laboratory engineered mutants containing defects in a predetermined polymerase gene. Several PB2 DNA recombinants were used to construct plasmid vectors for expression of PB2 in permissive cells. For lytic infection of primate cells SV40-PB2 recombinant DNA was successfully propagated in the presence of a helper SV40 early function mutant. Dot-blotting and "Northern" blotting mRNA analyses are currently being carried out to determine whether the PB2 protein encoded by the recombinant is functionally active. For persistent expression we employed a cloned mutant dihydrofolate reductase (DHFR) gene as a selectable marker. These studies have yielded several cell populations that survived drug selection. These cells are being analyzed for their ability to complement influenza mutants defective in PB2 function.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00407-02 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Persistent Expression of Cloned Influenza Genes in Permissive Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Kevin Ryan, Ph.D. | Staff Fellow | LID, NIAID |
| | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| | Robert Chanock, M.D. | Chief | LID, NIAID |

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| 1.2 | 1.0 | 0.2 |

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We sought to produce persistent expression of influenza virus cloned DNA in cells permissive for virus infection. Such cells would be useful for investigation of the molecular biology of influenza virus and for isolation of specific viral mutants via complementation by the expressed gene. In this manner, naturally occurring or laboratory engineered mutants containing viable deletion mutations could be isolated for evaluation of their level of attenuation. For selection of cells expressing transfected viral DNA sequences we employed an expression vector containing a mutant dihydrofolate reductase (DHFR) gene as a selectable marker. Alternatively, a neomycin resistance gene was used for coinfection and transformed cells were selected in the presence of G418, an analogue of neomycin. A series of recombinant DNA molecules were constructed between the DHFR expression vector and influenza nucleoprotein (NP) DNA under the control of an inducible metallothioneine promoter or a constitutive SV40 promoter. Transfection of simian CV-1 cells with these DNA recombinants followed by selection with methotrexate yielded cloned cell populations which were analyzed for NP synthesis. NP expressing cells were isolated at high frequency only when the inducible metallothioneine promoter was employed. Our failure to obtain NP expressing cells in the constitutive system suggests NP synthesis may be toxic to cell growth. Similar studies with influenza non-structural protein (NS) recombinants showed that stable synthesis of NS occurred only when the inducible system and the neomycin-resistant gene were employed. The stability of both the NP and NS producing cells is currently being analyzed. The expressed influenza virus protein in these cells will be analyzed for its ability to complement mutants that have a defect in that protein.



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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00408-02 LID |
|---|---|

PERIOD COVERED
October 11, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Attempts at Allele Replacement of NA Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Lewis J. Markoff, M.D. | Medical Officer | LID, NIAID |
| Others: | Ching-Juh Lai, Ph.D. | Head, MVB Sect. | LID, NIAID |

COOPERATING UNITS *(if any)*

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| 0.5 | 0.3 | 0.2 |

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(Dr. L. Markoff was on study leave at The Johns Hopkins Hospital, Department of Internal Medicine, Division of Infectious Diseases, during Oct. 1984 through June, 1985.)



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00409-01 LID

PERIOD COVERED

October 1, 1983 to September 30, 1984 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning and Expression of Influenza Virus Polymerase Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| Co-PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| Others: | Lewis Markoff, M.D. | Medical Officer | LID, NIAID |

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20205

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


We have cloned a full-length copy of double stranded DNA that codes for an influenza virus polymerase protein, PB2. PB2 is one of the two basic proteins that are present in small copy number in the viral nucleocapsid. PB2 binds and then cleaves capped host cell mRNA. This represents the first step in the priming of influenza viral mRNA transcription. We have initiated attempts express functional PB2 in mammalian cells. Initially, cloned PB2 DNA flanked by Bam HI linker sequences was inserted into the late region between the Hpa II and the Bam HI sites of an SV40 vector that contains a viable deletion in the small t region; this deletion provides additional room for packaging foreign DNA in SV40. Recombinant PB2-SV40 DNA was used for transfection of primate cells and was successfully propagated in the presence of an early SV40 <u>ts</u> mutant helper. Synthesis of the PB2 polypeptide in recombinant infected cells is currently being analyzed by immunoprecipitation and by <u>in vitro</u> translation of PB2 specific mRNA. A recombinant that expresses polymerase PB2 should be useful for determining whether PB2 by itself exhibits the functional activity that has been ascribed to it as a component of the nucleocapsid transcriptase complex. In addition, complementation analysis of <u>ts</u> influenza mutants will be carried out to test the biologic activity of PB2 produced in recombinant infected cells.


THIS PROJECT WAS COMBINED WITH Z01 AI 00369--03
TERMINATED



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00458-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Variation Among Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |
| Others: | Bangti Zhao, Ph.D | Visiting Associate | LID, NIAID |
| | Yoshihiro Makino, M.D. | Visiting Associate | LID, NIAID |
| | Erich Mackow, Ph.D. | Staff Fellow | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |

COOPERATING UNITS *(if any)*

Department of Virus Diseases, Walter Reed Army Inst. of Research, Washington, D.C. (Drs. Walter Brandt, Don Burke)

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| 1.0 | 0.8 | 0.2 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dengue virus family contains 4 distinct serotypes that are distinguishable by virus neutralization. Among the dengue group the type 2 virus is most frequently involved in hemorrhagic fever, a severe and often fatal form of dengue disease. There is considerable polymorphism among type 2 viruses as indicated by variation in oligonucleotide fingerprints and this variation has a geographic distribution in that specific patterns are limited to specific localities. Efforts are now underway to study genetic variation of dengue viruses by molecular cloning and nucleotide sequencing. Dengue 2 (strain PR159) was chosen for sequence comparison with dengue 4 that is also being cloned and sequenced in our laboratory. Dengue 2 genomic RNA was purified and transcribed into RNA-cDNA hybrids for direct cloning in the pBR322 vector according to the procedure established for dengue 4. Analysis of plasmid DNA after Pst I digestion on agarose gel showed that a majority of recombinant plasmids contained DNA inserts ranging from 500-4,000 base pairs in length. Recombinants with the largest inserts (2,000 base-pairs or more) were chosen for mapping the full-length genomic sequence. For this purpose we took advantage of the genetic homology that exists between dengue virus type 2 and type 4. Radio-labelled probes prepared from cloned DNA segments of dengue 4 at various map positions were used for initial mapping and screening of dengue 2 inserts. Thus far, more than one-half of the dengue 2 genomic sequences have been cloned and our ultimate goal is to obtain a full-length DNA copy. Complete sequence analysis will be performed in an effort to gain a better understanding of the pattern of virus polymorphism in dengue epidemics and the involvement of different virus strains in dengue hemorrhagic fever.



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|---|---|

PERIOD COVERED
October 1, 1984 to September 31, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Organization and Expression of Dengue Viruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Erich Mackow | Staff Fellow | LID, NIAID |
| Others: | Bangti Zhao | Visit. Assoc. | LID, NIAID |
| | Yoshihiro Makino | Visit. Assoc. | LID, NIAID |
| | Ching-Juh Lai | Head, MVB Section | LID, NIAID |

COOPERATING UNITS *(if any)*

Texas A & M University (Dr. M. Summers, Dept. of Entomology)

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 1.0 | 0.8 | 0.2 |

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The genome of dengue virus and other members of the flavivirus group consists of a positive strand 42S RNA 10-12,000 bases in length. A 42S mRNA produced in infected cells codes for the three structural proteins $V_1$, $V_2$, and $V_3$ which are also designated the envelope, core and matrix proteins, respectively. The remaining approximately 70% of the viral genome encodes a number of as yet unidentified non-structural proteins. The locations on the genome coding for the dengue structural and non-structural proteins have not been mapped and the functional role that each of these proteins plays remains to be determined.

Extension of cloned DNA by "genome walking" has thus far yielded dengue type 4 cDNA of 9 kilobases in 5 overlapping segments. The 5 clones are currently being extended in order to complete our cloning of the dengue genome. The clones are also being sequenced in order to study the gene organization of dengue virus. We plan to approach the mapping and protein analysis of dengue virus by using the recently developed baculovirus vector-host cell system for expressing cloned DNA segments. Expressed polypeptides will be used to immunize animals for preparation of antisera and the antisera will in turn be used for identifying viral proteins present in infected cells. In this manner, the expressed gene product will identify the viral structural or non-structural protein each gene encodes and allow us to map the position of each gene on the dengue genome. In addition, the antisera produced from expressed DNA segments should be useful for identifying the antigenic determinants of dengue virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00460-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification and Gene Mapping of Dengue Viral Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Yoshihiro Makino, Ph.D. | Visiting Associate | LID, NIAID |
| Others: | Bangti Zhao, Ph.D. | Visiting Associate | LID, NIAID |
| | Ching-Juh Lai, Ph.D. | Head, MVB Section | LID, NIAID |

COOPERATING UNITS (if any) Laboratory of Immunogenetics, NIAID (Dr. John Colligan).

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 0.8 | 0.6 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current information indicates that the flavivirus virion contains three protein components, i.e. the glycosylated envelope protein (E), the non-glycosylated matrix protein (M) and the capsid protein (C). These proteins are proteolytic products of a long polyprotein precursor that is translated from a genomic-length RNA species. The genes coding for these viral structural proteins are clustered at the 5'terminus. We sought to provide evidence that the dengue virus genome also contains one open-reading frame and that the encoded polyprotein is processed to yield the individual viral proteins found in the virion and in infected cells. Using polyclonal antisera for immunoprecipitation, we have identified three dengue virion components of 51Kd, 14Kd, and 8Kd respectively. These dengue-specific proteins are being prepared for determination of their amino-terminal amino acid sequence. In the meantime, cloned DNA segments located at the extreme 5'-end of the genome are being sequenced. These studies should enable us to determine the map positions of the genes that code for structural proteins. Also, the complete amino acid sequence of these structural proteins can be deduced from the cDNA sequence of their genes.



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GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00333-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
A Longitudinal Study of Viral Gastroenteritis in Infants and Young Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Albert Z. Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |
| Others: | Nilda Passarani, M.D. | Guest Worker | LID, NIAID |

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| <0.1 | <0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses have been studied extensively predominantly by cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. As reported previously, 139 rotavirus strains were detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgrouping pattern of tested strains was of special interest in that both subgrup 1 and subgroup 2 viruses were observed. In addition, as noted previously, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "O" agent. 150 (40%) of the children experienced at least one rotavirus infection; 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection. We will attempt to propagate selected rotavirus positive specimens in tissue culture by direct isolation or genetic reassortment in order to serotype them. Five specimens were serotyped directly from the anal swab specimen by solid phase immune election microscopy. Two were serotype 1, two were serotype 2 and one serotype 3.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00334-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Monoclonal Antibodies to Rotavirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Rebecca Tominack, M.D. | Medical Staff Fellow | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epid. Section | LID, NIAID |

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 0.4 | 0.2 | 0.2 |

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standerd unreduced type. Do not exceed the space provided.)


In order to devise a practical, quick assay for serotyping human rotavirus isolates, repeated attempts at isolating monoclonal antibodies directed at the major neutralization protein of human rotaviruses have been made. In the past, monoclonal antibodies directed at the outer capsid proteins $VP_3$, the hemagglutinin, and $VP_7$, the major neutralization protein, of RRV were isolated. Screening by hemagglutination-inhibition assay was of key importance in identifying monoclones directed at the outer capsid proteins of RRV. In order to isolate monoclonal antibodies directed at the major neutralization proteins of human serotypes 1, 2, and 4, Balb/C mice were immunized with human rotavirus x RRV reassortants which had human serotype specificity but contained the 4th gene (the hemagglutinin $VP_3$) and also the remaining genes from RRV. The mice were immunized approximately 3-4 times over a period of 2-4 months with partially purified virus. The fusion ratio was 10 spleen cells per NS-1 mycloma cell.

Screening of several fusions by hemagglutination-inhibition identified some monoclones which inhibited hemagglutination of the reassortant rotavirus but all of these monoclones were directed at the RRV 4th gene product, $VP_3$; none were directed at the human rotavirus $VP_7$ protein. A screening test involving neutralization of virus in 96 well tissue culture plates has been developed and will be used to screen for neutralizing antibodies to the different serotypes.

In addition, attempts are being made to develop an ELISA test which would enable serotyping of serotype 1 and 3 viruses by using monoclonal antibodies directed at the major neutralization protein of Wa (kindly supplied by Dr. Harry Greenberg) and at the major neutralization protein of RRV (which had been isolated in the past).




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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00335-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Rotavirus Reassortants: Genetics and Use in Rotavirus Vaccines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Rebecca Tominack, M.D. | Medical Staff Fellow | LID, NIAID |
| | Yasutaka Hoshino, D.V.M. | Visiting Assoc. | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epidemiology Sec. | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief | LID, NIAID |

COOPERATING UNITS (if any)

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.4 | 1.6 | 0.8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Reassortants with characteristics that make them potential vaccine candidates have been isolated from coinfection of primary tissue culture with fastidious human rotavirus (strains D, DS-1, P, or ST3, representing serotypes 1, 2, 3, or 4, respectively) and a wild type bovine or rhesus rotavirus. Analysis of the genotypes of these reassortants revealed that many contained 10 genes from the animal rotavirus parent and only one gene, that which codes for the major neutralization protein, $VP_7$, from the human rotavirus parent. Single human rotavirus gene substitution reassortants which have the human rotavirus neutralization specificity as determined by plaque reduction neutralization assay (PRNA) are available for each of the above coinfections (i.e., RRV x D, DS-1, or ST3; UK x D, DS-1, P, or ST3). These reassortants represent promising candidate live vaccine strains. Their animal rotavirus gene complement should attenuate them, but the major neutralization protein of human rotavirus should induce protective immunity. These single human rotavirus gene substitution reassortants have been adapted to growth in DBS-FRhL cells and 2 reassortants, DxRRV (6-1-1) and DS-1xRRV (240-2-1) have been prepared as vaccine lots at Flow Laboratories and are currently undergoing safety testing.

Careful neutralization studies of the reassortants described above have shown that a gene product in addition to $VP_7$ (coded by gene 8 or 9) is also involved in neutralization. This other gene product appears to be $VP_3$, coded for by the 4th gene. Attempts are being made to isolate reassortants which derive both their 4th and 8th or 9th genes from the human rotavirus parent but the remaining 9 genes from the animal rotavirus parent.




PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00338-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Rotavirus Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jorge Flores, M.D. | Expert | LID, NIAID |
| Others: | Roger Glass, M.D. | Medical Officer | LID, NIAID |
| | Mario Gorziglia, Ph.D. | Visiting Fellow | LID, NIAID |
| | Yolanda Aguirre, B.S. | Guest Worker | LID, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.4 | 0.8 | 1.6 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


We have obtained rotavirus cDNA clones from several genes of various rotavirus strains. They include the human strains Wa, DS1, Price, M-37 and ST3 and animal strains NCDV, RRV, UK and OSU.

Either single-stranded (ss) or double-stranded (ds) rotavirus RNA was employed as template for reverse transcription and cDNA synthesis; after second strand synthesis the cDNAs were introduced into pBR322 by the dC:dG tailing method. Transformation with the rotavirus/pBR322 recombinant plasmids yielded a large number of clones carrying rotavirus gene copies. The rotavirus gene segment cDNA present in the clones was identified by Northern blot hybridization or colony hybridization with cDNAs of known gene origin.

Clones carrying copies of the genes encoding the outer capsid hemagglutinin (gene 4) from the simian RRV and the bovine UK rotavirus have been identified and the RRV gene 4 has been partially sequenced. Clones with copies of the $VP_7$ glycoprotein gene of the animal rotaviruses OSU, NCDV, RRV and several human rotavirus strains have been identified, characterized by restriction mapping and some have been sequenced partially or in their entirety.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00339-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and Serotypic Characterization of Human and Animal Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principel Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yasutaka Hoshino, D.V.M. Visiting Associate LID, NIAID

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.9 | 0.3 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is designed to cultivate, directly in cell cultures, a variety of human and other animal rotavirus strains from diverse geographical areas and populations in order to define serotypic diversity and similarity and to select and develop potential vaccine candidates. The newly established serotype 4 rotaviruses have been shown to play an etiological role in acute gastroenteritis, and its worldwide distribution was also documented. It was shown that there is no correlation between "family" classification based on RNA-RNA hybridization and serotype designation based on neutralization. Two human rotavirus isolates from other laboratories, which are said to represent new serotypes, as well as a bovine rotavirus isolate which is suspected of belonging to serotype 2 are under investigation in our laboratory.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00340-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Experimental Studies in Animals with Various Rotaviruses and Their Reassortants

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| Others: | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |

COOPERATING UNITS (*if any*)

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.2 | 0.2 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


The aims of this project are to evaluate virulence and immune response of selected human and animal rotaviruses in experimentally infected animals, and to study cross-protection between selected strains of human and animal rotaviruses. Study of potential vaccine strains in experimental animals also constitutes an additional test for the presence of adventitious agents.

Experimental animal systems that are currently available and that have been shown to be suitable for the study of rotavirus infection and in some cases disease, are the colostrum-deprived newborn rhesus monkey and the gnotobiotic piglet. Chimpanzees, rhesus monkeys and African green monkeys are also utilized to examine the safety of potential rotavirus vaccine preparations. Piglets were delivered by Caesarean section and maintained in plastic isolators under gnotobiotic conditions; monkeys were maintained under strict isolation in contained units.

Sera and fecal samples derived from the animal studies were analyzed for antibody and antigen by a variety of laboratory techniques. Enzyme immunoassay was used to evaluate fecal samples for antigen, while complement fixation, immune adherence hemagglutination assay and/or plaque reduction neutralization assay were used to evaluate sera and fecal samples for antibody response.




PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00341-04 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Evaluation of Experimental Rhesus Rotavirus Vaccine in Infants and Children

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Albert Z. Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |
| Others: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Roger I. Glass, M.D. | Medical Officer | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |

COOPERATING UNITS (*if any*) U. of Md., (M. Levine, R. Black, M.L. Clements); Flow Labs., (L. Potash); U. of Rochester (R. Dolin); Vanderbilt U. (Dr. Wright); Marshall U. (Drs. Belshe, Anderson); U. of Tampere (Dr. Vesikari); U. of Umea (Dr. Gothefors); Ntl Inst. of Dermatology, VZ. (Dr. Perez-Schael).

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|---|---|---|
| 4.5 | 1.1 | 3.4 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Diarrheal disease is an important cause of morbidity in infants and young children in developed countries and a major cause of morbidity and mortality in the same age group in developing countries. A major goal of this laboratory is the development of a rotavirus vaccine to prevent serious rotavirus disease in infants and young children. Two approaches that have been evaluated in humans: 1) the use of a live attenuated human rotavirus strain and 2) the use of a live rotavirus strain of animal origin. With reference to the first approach, we had observed that the tissue culture adapted mutant of human rotavirus (HRV) serotype 1 (Wa strain) was antigenic and attenuated in adult volunteers. The second approach, employing an animal rotavirus as vaccine has been applied successfully by Vesikari, et al., using bovine rotavirus NCDV which was shown to be attenuated and to induce resistance to moderate or severe rotavirus diarrhea in infants and young children.

We have performed extensive studies with another animal rotavirus, rhesus rotavirus (RRV) strain MMU18006, which was adapted to diploid simian FRhL-2 cells and which shares protective antigens with human rotavirus serotype 3. In addition, rhesus rotavirus (RRV) appears to be restricted in humans because it has not been recovered from persons undergoing rotavirus infection under natural conditions. In volunteer studies that began in adults and progressed stepwise to infants 4 months of age, RRV was found to be quite antigenic. However, transient fever and loose stools were observed in young vaccinees less than 1 year of age who received $10^6$ pfu of RRV. However, infants 4-11 months old who were fed a $10^5$ or $10^4$ pfu of RRV in Venezuela did not develop fever or diarrhea. $10^5$ pfu of RRV induced rotavirus antibodies in the serum of 82% of vaccinees. Further studies with this vaccine are planned.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00342-04 LID |
|---|---|

PERIOD COVERED
October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Gastroenteritis Viruses by Electron Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Albert Z. Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |
| Others: | Nilda Passarani, M.D. | Guest Researcher | LID, NIAID |

COOPERATING UNITS *(if any)*

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| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Despite the development of 2nd and 3rd generation tests, EM remains an essential tool: (1) as the "supreme court" when newer tests yield variable results; (2) in the search for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of antibody to the virion in antigen-antibody reactions; (4) for serologic studies; (5) for direct visualization and fine structure characterization of the morphology of virus particles; and (6) for studying specimens derived from individuals with diseases of unknown etiology such as non-A, non-B hepatitis by immune electron microscopy. A new technique was introduced during the past year - solid phase immune electron microscopy - which was used successfully for the rapid serotyping of human and animal rotaviruses. In addition, the technique appeared to be more sensitive than conventional immune electron microscopy for rotavirus detection. Finally, since over 50% of the episodes of pediatric diarrhea are without known etiology, EM should continue to prove to be a valuable tool in the search for such agents.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00343-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Norwalk-Like 27nm Virus Particles in Viral Gastroenteritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epid. Section | LID, NIAID |

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|---|---|---|
| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Marin County agent is a 27nm virus-like particle which was associated with two separate outbreaks of nonbacterial gastroenteritis in northern California in 1978 by L. Oshiro. The agent is morphologically similar but serologically distinct from the Norwalk, Hawaii and Snow Mountain agents as assessed by immune electron microscopy (IEM) or solid phase radioimmunoassay (RIA) antibody blocking assay. One ml of a safety tested, bacteria-free filtrate prepared from a stool specimen from one of the individuals ill during the original Marin County outbreak was administered orally to seventeen adult volunteers. None of these individuals developed definite clinical illness. Two additional volunteers later received a 20ml inoculum. One of these volunteers developed a gastrointestinal illness characterized by nausea, vomiting, diarrhea and malaise. Interestingly, this illness started five days after administration of the fecal filtrate and lasted 36-48 hours. Examination by IEM of several diarrheal stool specimens from this volunteer demonstrated a large number of 27nm particles. These particles were shown to be identical to the Marin County agent in IEM studies using acute and convalescent sera from the original outbreak. A preliminary survey of a series of gastroenteritis outbreaks using a recently developed RIA failed to implicate the Marin County agent as an important cause of epidemic gastroenteritis.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00346-04 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetic Characterization of Rotavirus by Hybridization Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| Others: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| | Irene Perez-Schael, M.Sc. | Guest Worker | LID, NIAID |

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.2 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


We have used rotavirus single stranded (ss) RNA prepared by in vitro transcription from viral cores as well as cloned rotavirus cDNA as probes to investigate the molecular epidemiology of human rotaviruses and to assess the extent of genetic diversity among human and animal rotavirus strains.

The studies carried out thus far suggest that:

1) Genetic variation in human rotaviruses is rather common.

2) Rotavirus strains isolated from individuals of the same animal species show a greater extent of homology among themselves than with strains derived from other species.

3) Rotaviruses undergo genetic variation by either of two mechanisms: a) accumulation of successive sequence changes within specific genes (genetic drift); and b) gene reassortment (genetic shift) that results in the appearance of rotavirus strains with a constellation of genes which are derived from two or more distinct rotaviruses. The relative importance of these two mechanisms (genetic drift or shift) in the generation of new strains is not known. Partial sequence analysis of nosocomial rotavirus strains recovered from neonates who underwent asymptomatic infection suggests that the rotavirus genome does not have a high rate of spontaneous mutation; on the other hand, rotavirus strains have been identified that appear to have been derived by gene reassortment.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00373-02 LID

PERIOD COVERED

October 1, 1984 through September 30, 1985 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies for Detection of Etiologic Agent(s) of AIDS by Immune Electron Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Albert Z. Kapikian, M.D Head, Epidemiology Section LID, NIAID

COOPERATING UNITS *(if any)* National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, Maryland (Drs. Gravelle, London, Sever)

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Epidemiology Section

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.0 | 0.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acquired immune deficiency syndrome (AIDS) is a serious disease of major public health importance. Immune electron microscopy, which is the direct observation of antigen-antibody interaction, has been employed as a means for detecting the fastidious etiologic agents of several diseases such as the 27 nm Norwalk agent of acute nonbacterial gastroenteritis, and the 27nm hepatitis A virus of hepatitis. Collaborative studies using electron microscopy were carried out on simian AIDS specimens in collaboration with the NINCDS. A retrovirus-like agent was visualized by negative stain electron microscopy.

T E R M I N A T E D



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00410-02 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Binding of Rotavirus to Cell Surface Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |

COOPERATING UNITS *(if any)*

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In an attempt to characterize the nature of the receptors on cell surfaces and of the viral protein involved in this initial interaction between rotavirus and cells, several approaches have been followed. (1) Enzymatic treatment of human type O erythrocytes before utilization in hemagglutination assay has resulted in the separation of rhesus and bovine (NCDV strain) rotaviruses into one group and two avian rotavirus isolates into another group with respect to their ability to agglutinate these enzymatically treated erythrocytes. (2) Non-hemagglutinating human rotaviruses have been shown to bind to human erythrocytes in a modified radioimmunoassay using the erythrocytes as solid phase. (3) Labeled rotavirus has been shown to react with membrane proteins isolated from both the microvillus of enterocytes of the small intestine of pigs as well as from human erythrocytes. (4) Rotavirus was demonstrated to bind to glycosphingolipids in a thin layer chromatography system. (5) The viral protein involved in the initial binding to MA 104 cells has in preliminary experiments been found to have a molecular weight of approximately 20,000 daltons. Attempts to produce monoclonal antibodies against membrane proteins isolated from enterocytes as well as from erythrocytes have been carried out. Production of monoclonal antiidiotypic antibodies against the protein encoded by gene 4 or gene 9 of rhesus rotavirus has also been attempted.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00411-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985 TERMINATED

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relative Frequency of Rotavirus Subgroups 1 and 2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jorge Flores, M.D. Visiting Scientist LID, NIAID

COOPERATING UNITS *(if any)*

Instituto Nacional de Dermatologia, Instituto Nacional de Nutricion, Caracas, Venezuela

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 0.0 | 0.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies recently developed against the 42,000-dalton protein of two rotavirus strains were used in an enzyme-linked immunosorbent assay to determine the subgroup specificity of rotaviruses obtained from Venezuelan children with rotavirus gastroenteritis. Subgroup 2 rotavirus was shed by 85% of the children, whereas only 14% shed subgroup 1 rotavirus. No differences were found in the occurrence of fever and vomiting between chidren shedding either rotavirus subgroup, but it appeared that the syndrome tended to last longer in children shedding subgroup 2 rotavirus.

TERMINATED



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00461-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Serial Passages of Bovine and Rhesus Rotaviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |
| Others: | Mario Gorziglia, Ph.D. | Visiting Fellow | LID, NIAID |
| | Karen Midthun, M.D. | Med. Staff Fellow | LID, NIAID |
| | Yasutaka Hoshino, D.V.M | Visiting Associate | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |

COOPERATING UNITS *(if any)*

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| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Genomic rearrangement of bovine rotavirus (NCDV strain) has been found following serial passage in cell culture at initial high multiplicity of infection. Plaque purified virus from passage 14 contained dsRNA with changes in electrophoretic pattern. Gene segment 5 could not be detected while a new band migrating between RNA segments 1 and 2 appeared. In a hybridization experiment homology was observed between gene segment 5 and the new band. Differences in the production of viral proteins between the two viruses could not be detected.

Attempts to demonstrate (1) interference between these viruses and (2) genomic rearrangement in other serotypes are in progress.





PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00462-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Rotavirus Vaccine Field Trial in Venezuela

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: Jorge Flores, M.D. Visiting Scientist LID, NIAID

COOPERATING UNITS (*if any*)

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.2 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We are currently investigating the potential usefulness of the rhesus rotavirus MMU18006 strain developed as a vaccine candidate at the Laboratory of Infectious Diseases (NIAID). This virus is a simian rotavirus strain derived from a stool of a 3.5 month old rhesus monkey with acute diarrhea. In the phase I study the vaccine was administered in two different doses ($10^5$ or $10^4$ pfu which represented a 1/100 or 1/1000 dilution of RRV-1 respectively), to 35 infants (4-10 months old). A third group comprised 18 infants received placebo. The study was carried out in a double-blind fashion. The infants were examined daily for presence of side reactions. Rectal temperature was taken twice a day by medical personnel and stool specimens were collected daily and analyzed for rotavirus shedding. Significant reactions were not observed in the infants who received either dose of the vaccine when compared to the placebo group. A low incidence of fever, loose stools (including 3 cases of diarrhea) and upper respiratory symptoms were observed; however, these symptoms and signs were as common in the placebo group as in the immunized children. We have recently examined the serum samples obtained from the 53 children. Serological assays performed for rotavirus antibody included complement fixation assay, immune adherence hemagglutination assay, plaque reduction neutralization assay and a tube neutralization assay. Overall, 65% of the vaccinated children developed a seroresponse; 76.5% of the children receiving the high dose (1:100 dilution of the original vaccine stock) had a response compared to 53% of those receiving the lower dose (1:1000 dilution). For the phase II study, approximately 100 infants will be vaccinated. 100 additional infants will receive placebo in a double-blind fashion.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00463-01 LID

PERIOD COVERED
September 1984 to September 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Oligonucleotide Probes for Detection and Identification of Rotavirus Serotypes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Roger Glass, M.D. | Medical Officer | LID, NIAID |
| Others: | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| | Jerry Keith, Ph.D. | Section Chief | Rocky Mtn Lab, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID/NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.3 | 0.3 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Labelled oligonucleotides are being used extensively as genetic probes because they are relatively easy to make and under ideal conditions, can descriminate by hybridization between gene sequences that differ by a single base pair. The recent proliferation of sequences for the neutralizing gene of different strains of rotaviruses encouraged us to prepare oligonucleotide probes to a series of areas common to all serotypes of rotavirus as well as to areas demonstrating great serotypic diversity. We have tested these probes for their sensitivity and specificity in detecting rotavirus in stool specimens and in serotyping those specimens found to be positive. We have also compared the sensitivity of short oligonucleotide probes with larger single stranded RNA transcipts prepared according to procedures developed previously in this laboratory. Radiolabeled oligonucleotide probes appear to be about as sensitive as the ELISA test in detecting rotavirus in stool specimens and are significantly less sensitive than ssRNA transcript probes. The level of sensitivity and specificity are reduced when biotinylated oligonucleotide probes are used. Serotyping will be examined by hybridizing oligo probes to Northern blots of viral RNA since neither specificity nor sensitivity were adequate using dotted stool RNAs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00448-01 LID

PERIOD COVERED
November 1, 1985 to June , 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rotavirus Vaccine Trial in Umea, Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Roger Glass, M.D. | Medical Officer | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epidemiology Sect. | LID NIAID |

COOPERATING UNITS *(if any)*

University of Umea, Umea, Sweden (Dr. Goran Wadell, Dr. Leif Gothefors)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.4 | 0.3 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


A field trial of the rhesus rotavirus (RRV) vaccine was begun in Umea, Sweden in January 1985 to examine the efficacy of the vaccine given at a dose of $10^6$ pfu. 106 infants 4-12 months of age were given placebo or vaccine with a bicarbonate-citrate buffer at the start of the rotavirus season. These individuals will be followed actively for rotavirus diarrhea and serologic responses for 18 months.

In the first month of followup, vaccinees had a significantly greater number of low grade fevers and loose stools than placebo recipients. Consequently, 2 phase I trials are being planned before recruitment of more infants into the trial continues. In one trial, reactogenicity of a lower dose of vaccine and the importance of the buffer will be examined by giving groups of 15-20 infants aged 4-12 months a lower dose of the vaccine with the bicarbonate-citrate buffer and without buffer. A third group will receive placebo alone. In the second phase I trial, immunogenicity and reactogenicity of the lower dose vaccine will be examined among infants 1 month of age.

In the field trial, about 40 infants have had diarrhea during the first rotavirus season so this trial could potentially be the first to establish efficacy of the RRV vaccine.




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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00449-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies Examining a Protective Role for Breast-feeding in Rotavirus Diarrhea

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Roger Glass, M.D. | Medical Officer | LID, NIAID |
| Others: | Albert Z. Kapikian, M.D. | Head, Epidemiology Sect. | LID, NIAID |
| | Richard G. Wyatt, M.D. | Special Assistant for Intramural Affairs | LID, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To investigate whether breast-feeding protects children against rotavirus diarrhea (RVD), we compared rates of breast-feeding by age and enteric pathogens among 2276 children 0-4 years of age who attended a diarrhea hospital in Bangladesh. Infants 0-5 months were less likely to be breast-fed than children 6-11 months of age suggesting that some protection was associated with early breast-feeding. In every age group studied, breast-feeding was more common among children with RVD than those children with non-RVD whereas it was less common among children with cholera and shigellosis. Twenty percent of breast milks had high levels of neutralizing activity (≧320) to the human Wa strain of rotavirus but among infants less than 1 year, this activity did not appear to be protective since the 30 infants with RVD consumed milk whose titer did not differ significantly from 44 infants with diarrhea of other cause. Despite the prolonged breast-feeding which is common in Bangladesh, the mean age of hospitalization with RVD is approximately the same as in countries where the duration of breast-feeding is quite short. None of these 3 independent observations support a protective role for breast-feeding among children against rotavirus diarrhea after the first months of life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00450-01 LID

PERIOD COVERED

October 1, 1984 to September 30,1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sequences and Expression of Gene 9 from Porcine Rotavirus Strain OSU

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Mario Gorziglia, Ph.D. | Visiting Fellow | LID, NIAID |
| Others: | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Yolanda Aguirre | Guest Worker | LID, NIAID |

COOPERATING UNITS *(if any)*

Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

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NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 1.7 | 1.7 | 0.0 |

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A full size cDNA copy of gene 9 (the gene that codes for the outer capsid glycoprotein VP7) from porcine rotavirus strain OSU has been cloned and its restriction pattern analyzed. We are in the process of sequencing this gene and transferring it to expression vectors. We are employing the expression vector system developed by Dr. Inouye at Stoneybrook University. Currently, different clones of the vectors containing copies of gene 9 have been constructed and are being analyzed for expression.



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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00451-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rotavirus Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| Others: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |

COOPERATING UNITS (if any)

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|---|---|---|
| 1.4 | 0.5 | 0.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The genome of the rotaviruses which belong to the reoviridae family, consists of 11 discrete segments (genes) of ds RNA. Its unusual segmented genome structure and the relative ease with which reassortants can be generated *in vitro* provides a unique opportunity to gain insight into genetic and molecular mechanisms of virus-host interactions through mapping biological function to specific genes. During the course of characterizing a variety of rotaviruses of mammalian and avian origin for serotype by plaque reduction neturalization, several intertypic rotaviruses were identified. During the past year, efforts were made to dissect genetically and delineate, serologically, the basis for this "bridging" phenomenon. The fourth gene product, protein VP3, was shown to be responsible for intertypic "bridging" in several instances. Thus, in addition to VP7, the VP3 protein contains antigenic sites which stimulate and react with neutralizing antibodies. In addition, antigenic and functional analysis of the rotaviral fourth gene product was performed.



PHS 6040 (Rev. 1/84)

GPO 914-918

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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00452-01 LID

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Characterization of Rotaviruses from Asymptomatic Human Neonatal Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


| | | | |
|---|---|---|---|
| P.I.: | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| Others: | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |


COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Infectious Diseases

SECTION
Epidemiology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 0.2 | 0.1 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Nineteen rotavirus strains derived from asymptomatic neonates (seven from England, five from Australia, two from Venezuela, and five from Sweden) were successfully cultivated in primary African green monkey kidney cell cultures, serotyped by plaque reduction neutralization (PRN) assay, subgrouped by indirect enzyme-linked immunosorbent assay, and electropherotyped by polyacrylamide gel electrophoresis. All 19 strains were shown to fall into one of the four known human serotypes; serotype 1 (all Venezuelan strains), serotype 2 (all Swedish strains), serotype 3 (all Australian strains), or serotype 4 (all English strains). Hyperimmune guinea pig antiserum raised against the Venezuelan strain (M37) neutralized not only serotype 1 (strain Wa) but also serotype 4 (strain St. Thomas no. 3) viruses to a similar degree. The English, Australian, and Venezuelan isolates were found to belong to subgroup 2, and the Swedish strains were subgroup 1 viruses.





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**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00453-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vaccinia Recombinant Containing Bovine Rotavirus Glycoprotein Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Osamu Nakagomi, M.D., Ph.D. | Visiting Fellow | LID, NIAID |
| Others: | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Toyoko Nakagomi, M.D., Ph.D. | Guest Worker | LID, NIAID |
| | Yasutaka Hoshino, D.V.M. | Visiting Associate | LID, NIAID |
| | Robert M. Chanock, M.D. | Chief, LID | LID, NIAID |
| | Albert Z. Kapikian, M.D. | Head, Epid. Sect. | LID, NIAID |

COOPERATING UNITS *(if any)*

LVD, NIAID (Dr. Bernard Moss, Dr. Geoffrey Smith)

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

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|---|---|---|
| 1.0 | 1.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


We constructed a vaccinia virus recombinant which expressed bovine rotavirus glycoprotein VP7 (the major neutralization protein) by inserting a cDNA copy of the 9th gene from NCDV into the TK gene of vaccinia virus. This recombinant virus expressed a polypeptide of approximately 35,000 dalton which migrated closely with VP7 of NCDV. We vaccinated two rabbits with this recombinant virus intradermally and observed their response by various serological methods. Immunofluorescent and plaque reduction neutralization tests showed a significant increase in titer following vaccination although relatively high levels of pre-existing antibody to rotavirus made proper interpretation difficult.

This preliminary experiment demonstrated the necessity of using animals lacking rotaviruses antibody in further animal experiments to determine the antigenicity of this recombinant. We screened sera from cotton rats, mice, hamsters, guinea pigs and rabbits by neutralization assay. Although many of the animals had rotavirus antibody, it appeared that, if a sufficient number of mice, hamsters, or guinea pigs were used, a small proportion should have little, if any antibody. Thus, mice and hamsters have been inoculated with the recombinant vaccine virus to study their serological response to the NCDV VP7 component of the vaccinia-rotavirus VP7 recombinant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00454-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression of Bovine Rotavirus Glycoprotein Gene in Mammalian Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Osamu Nakagomi, M.D., Ph.D. | Visiting Fellow | LID, NIAID |
| Others: | Toyoko Nakagomi, M.D., Ph.D. | Guest Worker | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |

COOPERATING UNITS (if any)

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Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

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|---|---|---|
| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A full-length complementary DNA copy of the 9th gene of the bovine rotavirus NCDV strain which codes for a glycoprotein that induces neutralizing antibody was cloned into the late region of pSV2330, a hybrid expression vector that includes pBR322 plasmid DNA sequences, the simian virus 40 (SV40) early region and SV40 late region promoters, splice sequences, polyadenylation sequences and transcription termination sites. A near full-size cDNA copy of NCDV gene 9 which lacks the first but not the 2nd translation initiation codon is also ready to be cloned into pSV2330. This pSV2330 - monkey kidney cell system has proven by other researchers in this laboratory to be useful for studying influenza viral proteins that must be post translationally modified to achieve their biological activity. Partly based on their experience, we will first examine the antigenicity of the recombinant protein product by monoclonal antibodies and then ask whether either glycosylation or N-terminal hydrophobic region plays a role in its localization within the transfected cells. The findings which will be obtained in this study would broaden our understanding of rotavirus infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00455-01 LID

PERIOD COVERED

October 1, 1984 through September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of Rotavirus Neutralization Protein in Bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*



| | | | |
|---|---|---|---|
| P.I.: | Toyoko Nakagomi, M.D., Ph.D. | Guest Worker | LID, NIAID |
| Others: | Osamu Nakagomi, Ph.D. | Visiting Fellow | LID, NIAID |
| | Jorge Flores, M.D. | Visiting Scientist | LID, NIAID |
| | Jon Askaa, D.V.M. | Visiting Fellow | LID, NIAID |



COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*



We have attempted to express rotavirus antigen in E. coli as a means of developing an effective and safe rotavirus vaccine. If such an antigen is located on the bacterial surface it may stimulate local immunity by colonizing the small intestine. Toward this goal, we have utilized an open reading frame (ORF) expression vector (pORF2) which may direct the expression of rotavirus gene segments. The insertion of rotavirus cDNA sequences in this vector may allow the expression of hybrid proteins which could be transported to the cell surface.

When we cloned various sets of Sau 3A partial digests of NCDV and RRV gene (encoding VP7) cDNA into pORF2, only a few of these constructs expressed recombinant molecules, although the in-frame insertion of the gene segments into pORF2 had been achieved. The highest levels of expression (up to 14% of E. coli protein) were achieved with the shorter segments, however the resulting hybrid proteins tested by immunoprecipitation were not recognized by either polyclonal or monoclonal antisera. When we cloned larger fragments of the NCDV VP7 gene the level of expression was not high enough to allow further studies with this system. We also made constructs in which a λ PL promoter fragment was introduced 5' upstream of the fusion genes instead of ompF promoter originally provided with pORF2 vector. The level of expression achieved with this stronger promoter was, however, not significantly increased.

The PL promoter has also been used to attempt expression of defined segments of the NCDV gene 9 (VP7 gene). One such construct (carrying 822bp coding sequence) directed the expression of a protein of ±28,000 daltons when transformed into E. coli. Immunological examination of this protein is in progress.





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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00456-01 LID

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Assay of Coproantibodies to Rotavirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Rebecca Tominack, M.D. | Medical Staff Fellow | LID, NIAID |
| Others: | Karen Midthun, M.D. | Medical Staff Fellow | LID, NIAID |
| | Albert Kapikian, M.D. | Head, Epid. Section | LID, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Infectious Diseases

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.2 | 0.2 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are attempting to develop a solid phase ELISA system for the detection and quantitation of immunoglobulins in human stool specimens directed againts rotavirus. Major efforts to date have been directed at establishing reliable and optimal procedures and reagents for each step in this multi-layered "sandwich" assay. The basic test system is: 1) precoat 96 well plates with hyperimmune goat 930 rotavirus antiserum as capture antibody 2) add rotavirus antigen (3) add test specimens of serum or stool containing antibodies to rotavirus (4) add antibodies to human immunglobulin conjugated to peroxidase and (5) add specific substrate for peroxidase which causes development of color that can be read as optical density units. To date several difficulties have been encountered. The most confounding difficulty has been the long unrecognized intermittent partial/total failure of the conjugate. One other problem is that of high background color due to non-specific interactions with the precoat and several modifications will be explored to overcome this difficulty including use of purified rotavirus antigen, treatment to block "nonspecific" sites, change in the order of the ELISA sandwich layers.



PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF MICROBIAL IMMUNITY
1985 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY, NIAID
October 1, 1984 to September 30, 1985

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

Multiple genes on different chromosomes influence to antibody response to bacterial polysaccharide antigens. The results of previous studies conducted in our laboratory showed that multiple genes influence the the magnitude of the antibody response of mice to Type III pneumococcal polysaccharide (SSS-III) and the 21-73 determinant group of bacterial (Leuconostoc) dextran. Studies conducted with B6.C congenic strains of mice, enabled us to assign these genes to specific chromosomes. They showed that genes located on chromosomes 1,3,4, 5 and 9 influence the magnitude of the antibody response to SSS-III, whereas genes on chromosomes 1,4 and 5 - along with genes on chromosomes 12 and 16 - influence the magnitude of the antibody response to dextran. Some of these genes appear to be antigen-specific in their mode of action since they effect the response to SSS-III, but not dextran. (Dr. P. J. Baker; P. W. Stashak, M. Fauntleroy, and G. Caldes, LMI, NIAID, NIH).

Functional defects in lymphocytes of autoimmune mice. It was found that enlargement of Lyt-2$^+$ T cells in aging NZB mice was highly correlated with the titer of anti-erythrocyte autoantibody and the degree of hemolytic anemia. Lyt-2$^+$ T cell enlargement did not occur in NZB.xid mice, suggesting that the polyclonal activation of the Ly-1$^+$ B cells, which is blocked by the xid gene, may be associated with the late-life alteration in Lyt-2$^+$ T cells. Interestingly, NZB B cells express IL-2 receptor as they age.

T suppressor function, assessed using the pneumococcal polysaccharide SSS-III system, declined with age. In addition, old NZB mice were resistant to adoptive transfer of competent T suppressor cells.

Genetic analysis of NZB-NFS recombinant-inbred mice reveals an association between polyclonal B cell activation early in life and subsequent development of autoimmune disease. Taken together, these observations suggest that age-related changes in T and B lymphocytes, resulting in loss of T suppressor function, are closely related to the development of disease in NZB mice.

In contrast to NZB mice, Lyt-2$^+$ T cell enlargement did not occur in (NZBxNZW)$F_1$ mice, which develop autoimmune immune complex glomerulonephritis rather than hemolytic anemia. Examination of tolerance to BSA showed that both NZB and (NZBxNZW)$F_1$ mice have defects in the T lymphocyte compartment. This abnormality is much more pronounced in the $F_1$ than the NZB parent. The resistance to tolerance is present early in life, whereas the loss of T suppressor function is delayed. Thus, differences in the age of onset (earlier in the $F_1$) and characteristics of disease, may be a consequence of the difference in the primary T cell abnormality: severe resistance to tolerance induction in (NZBxNZW)$F_1$ as opposed to loss of T suppressor function in the NZB parent. (Drs. Lal, McCoy, Chused, and Baker; Ms. Brown, LMI, NIAID).

New cytometric methods for measurement of membrane potential and intracellular calcium. Using a newly developed method of measuring membrane potential with oxonol dyes in the flow cytometer, it was found that T lymphocytes, but not B lymphocytes, possess a calmodulin-dependent, calcium-sensitive potassium channel. Lymphocytes and monocytes, but not granulocytes, buffer membrane potential over a greater than physiologic range of extracellular potassium levels.

In collaboration with Dr. Roger Tsien, a technique was developed for measuring intracellular calcium in the flow cytometer with a new dye, indo-1. It was found that $[Ca^{++}]$ is tightly controlled. Exposure of spleen cells to anti-immunoglobulin causes a rapid (20 second) release of $Ca^{++}$ from intracellular stores. Blocking the B cell IgG Fc receptor with the monoclonal antibody 24G2 prolonged this calcium transient and increases the enhanced Ia and cell size responses to anti-Ig. This is consistent with a relationship between the level of $[Ca^{++}]$ and the cellular response and suggests a molecular linkage between a membrane $Ca^{++}$ channel and the Fc receptor. (Drs. Wilson and Chused; Ms. Edison, LMI, NIAID.)

Proliferative responses *in vitro* of antigen specific B lymphocytes require lymphokines. We have investigated the nature of soluble factors which regulate antigen-specific B cell proliferation. Our data support and extend the previous distinction of Type 1, Type 2, and thymus-dependent (i.e. TD) antigens. Type 1 antigens (e.g. TNP-LPS) induced antigen-specific B cell proliferation in the absence of exogenously added growth factor. Type 2 antigens (e.g. TNP-Ficoll) showed an absolute requirement for exogenously added BSF-1 in the induction of antigen-specific B cell proliferation. Furthermore, this proliferation could be enhanced by recombinant IL-1 and totally suppressed by recombinant $\gamma$-IFN. By all of these criteria, the activation requirements of Type 2 antigens were identical to those previously ascribed to anti-IgM antibodies. Induction of antigen-specific B cell proliferation by TD antigens (e.g. TNP-OVA) showed an absolute requirement for carrier-specific helper T cells. Development of a carrier-specific T cell line which was incapable of BSF-1 production additionally revealed that BSF-1 was also an absolute requirement for the induction of specific B cell proliferation by such antigens. Carrier-specific TD responses may require 3 signals. (Mr. Stein; Drs. Howard, Dubois and Greenblatt, LMI, NIAID.)

Characterization of early precursors of T cells. Multiparameter flow cytometric analysis was used previously to identify a small (2-5%) subpopulation of thymocytes which were large and which expressed a small amount of Lyt1 and no Ly2 or L3T4 antigen on their membranes. These cells (dLy1 cells) could be isolated, and were shown in adoptive transfer studies to be the precursors of the 3 other major populations identified in thymus by flow cytometry. These cells appear to be the earliest intrathymic precursor of other T cells.

Purified dLy1 cells contain messenger RNA for the $\beta$ and the $\gamma$ chains of the T cell antigen receptor, but not of the $\alpha$ chain. Furthermore, somatic cell hybrids between purified dLy1 cells and thymic lymphoma produced some clones with rearrangement at the $\beta$ chain locus and some with the locus in the germline configuration, indicating heterogeneity in the dLy1 cells. Further heterogeneity was found with respect to expression of the receptors for IL-2, for the transferrin receptor, and the antigens TL and Pgp. (Drs. Fowlkes, Chused, Ms. Edison, LMI, NIAID; Drs. Lechler, Samelsen, Germain and Schwartz, LI, NIAID; Dr. Mathieson, NCI.)

Both disease and immune protection transferred with T lymphocytes in experimental autoimmune encephalomyelitis of guinea pigs. Experimental autoimmune encephalomeylitis (EAE) is a model for demyelinating diseases of man with its pathogenesis of pressing interest. The type of lymphocyte involved in inducing EAE of guinea pigs (the animal of choice for delayed type responses) was sought by current cellular approaches. Lymph node cells from strain 13 inbred guinea pigs immunized with isogeneic spinal cord in complete Freund's Adjuvant were separated by depletion of B cells ("panning") on petri plates coated with rabbit anti-guinea pig IgG antibody. This depletion was sometimes augmented by complement-mediated lysis using mouse anti-guinea pig B cell monoclonal antibody, rabbit anti-mouse Ig, and rabbit complement. The B cells separated by the panning technique did not transfer EAE, but the non-adherent population (mainly T cells), after stimulation with myelin basic protein in vitro, induced EAE in recipients. Flow cytometric analysis shows 87.2% of the non-adherent cells stain with anti-T monoclonal antibodies. Three x $10^7$ nonadherent cells induced severe adoptive disease. Sub-optimal ($1 \times 10^7$) numbers of non-adherent cells induced some resistance to active EAE in recipients, invoking suppressor cell involvement as a possible mechanism. B cells separated by panning did not provide protection against active sensitization with spinal cord antigen. The low histological scores of lesions in the adoptively-transferred EAE were in sharp contrast to the high clinical scores of these animals suggesting that the infiltrating cells seen in perivascular cuffs in the CNS were not necessarily those most responsible for the clinical manifestations. (Drs. Jarjour and Stone; Ms. Amsbaugh, LMI, NIAID.)

Human-mouse hybridoma services both IgG and IgM antibodies to tetanus toxoid. Human blood lymphocytes were immunized in vitro with denatured, immobilized tetanus toxoid (TT). Immunized cells were fused in vitro with the mouse myeloma line SP-2/0, and the somatic hybrids cloned by limiting dilution. One of the hybrids synthesized both human IgM and IgG, and the culture fluids contained antibody to TT. Thirty subclones of this hybrid were each secretors of IgM and IgG as well as anti-TT antibody. In each subclone, both human IgG anti-TT and human IgM anti-TT were found in high titer by ELISA test. Almost 100% of cells examined by immunofluorescence contained cytoplasmic IgG and IgM. This is the first example of a hybridoma or myeloma secreting two isotypes of the same antigen-specificity. (Drs. Chu and Asofsky, LMI, NIAID)

Administrative

The Laboratory was joined by Dr. George Wesley, a Medical Staff Fellow, Drs. David Ennist and Karen Elkins, Staff Fellows, and Dr. Yasuo Ishida, a Visiting Fellow. Drs. Carol Sulis and David Greenblatt concluded Medical Staff Fellowships, but remain as guest researchers. Peter Stein, a senior medical student at Johns Hopkins University spent a year in the Laboratory on an American Heart Association research grant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00131-18 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of hypersensitivity in inbred histocompatible guinea pigs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | S. H. Stone | Head, Experimental Autoimmunity Section | LMI, NIAID |
| Others: | W. Jarjour | Visiting Fellow | LMI, NIAID |
| | D. F. Amsbaugh | Biologist | LMI, NIAID |
| | M. B. Datiles* | | |
| | C. S. Raine** | | |
| | U. Traugott** | | |

COOPERATING UNITS *(if any)*

*Clinical Branch and Laboratory of Vision Research, NEI; **Division of Neuropathology, Albert Einstein College of Medicine, New York, NY

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Autoimmunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☐ (b) Human tissues
- ☑ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying autoimmune encephalomyelitis (EAE) in juvenile guinea pigs which undergo a chronic stage after the acute phase. Monoclonal anti-guinea pig T cells were used to trace T lymphocytes to CNS sites. We are now using such monoclonals to separate and characterize the T cells from guinea pig spleens, lymph node and peritoneal exudate using plating, panning and cell sorter to isolate populations. The significance of the project lies in the opportunity to use monoclonal antibodies (developed at NIAID by Dr. Shevach in mice against surface antigens of lymphocytes of guinea pigs) to identify subpopulations responsible for tuberculin reactivity and induction of autoimmune diseases. First, by panning and cell-mediated lysis, we showed that T cells and not B cells were involved in the induction of guinea pig EAE, just as in mice and rats. Although the antigens cannot yet be characterized, we used a two-color system in cell sorter analysis and two different monoclonal mouse anti-guinea pig T cell antibodies to isolate subpopulations tagged by one or the other of them. One subpopulation induced EAE in histocompatible recipients after purification by cell-mediated lysis using monoclonal antibodies and rabbit complement to remove inactive subpopulations from the T cell population.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00134-23 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of immunoglobulin synthesis in mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI : | R. Asofsky | Chief, Laboratory of Microbial Immunity | LMI, NIAID |
| Others: | C. Sulis | Medical Staff Fellow | LMI, NIAID |
| | D. Greenblatt | Medical Staff Fellow | LMI, NIAID |
| | T. Teranishi | Visiting Associate | LMI, NIAID |
| | A. Brooks | Bio. Laboratory Technician | LMI, NIAID |

COOPERATING UNITS (if any)

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.7 | 2.7 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TH 2.5 is a B cell hybrid which an be induced to secrete IgM by stimulation with LPS and anti IgM in the absence of lymphokines. Cells from this line were stimulated with LPS. Stimulated and unstimulated cells were subjected to limiting dilution analysis for their ability to form large clones. Induction with LPS reduced cloning efficiency at least 50-fold. Most cells still produced microscopic clusters which did not continue to grow. Those clones which grew vigorously in these conditions were subcultured and reanalyzed. No selection for resistance to LPS was observed; induced cells again showed a greatly reduced cloning efficiency when exposed to LPS. The results suggest that the induction of immunoglobulin secretion with LPS commits many of these cells to terminal differentiation. An as yet undetermined factor(s) seems responsible for protecting some cells from this commitment, but clones derived from such "protected" cells are phenotypically similar to or identical to the original hybrid.

We have obtained four B cell hybrids which respond to BSF-1. Two show an increased rate of growth, but no change in membrane Ia. Two others show increased expression of Ia antigens on the membrane, but no change in rate of growth.



PHS 6040 (Rev. 1/84)

GPO 904-917

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00136-13 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Differentiation of Thymic Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI : | B. J. Fowlkes | Microbiologist | LMI, NIAID |
| Others: | R. Asofsky | Senior Investigator | LMI, NIAID |
| | T. M. Chused | Senior Investigator | LMI, NIAID |
| | L. M. Edison | Biologist | LMI, NIAID |
| | S. F. Cheng | Student | LMI, NIAID |
| | H. Ton | Student | LMI, NIAID |

COOPERATING UNITS (if any)

B. Mathieson, FCRC, NCI; A. Kruisbeck, DCT, NCI; R. Germain, LI, NIAID; R. Schwartz, LI, NIAID

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 1.1 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several subpopulations of thymocytes have been identified and isolated from the mouse thymus. One of these subpopulations, designated dLy1, has been demonstrated by differentiation studies in vitro and in vivo to represent a very early stage in intrathymic development. These studies were supported by molecular analysis demonstrating that these cells expressed mRNA specific for gamma and beta but not alpha chain of the T cell antigen receptor. Heterogeneity for beta-chain rearrangements also supported this concept. The current studies have investigated the heterogeneity observed in dLy1 thymocytes by size, surface antigen expression, and beta-chain gene rearrangement. Preliminary results indicate that expression of the IL-2 receptor may correlate more closely to maturation state rather than lineage commitment.

A collaborative project has demonstrated that cortical-type thymocytes are the precursors to the L3T4$^+$, Lyt2$^-$ medullary-type thymocytes. In vivo treatment with antibodies to Ia, dendritic cells, for Lyt2 prevents the development of specific subsets of T cells. Thus several approaches have been of use and aided in elucidating the T cell developmental sequence and lineage relationships.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00141-10 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune responses to malaria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Langhorne Visiting Fellow LMI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0 | 0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Suspended.

Continuing aspects of this project are included in: Z01 AI 00134-23 LMI.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00143-16 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic control of the antibody response to microbial antigens.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | P. J. Baker | Head, Microbiology and Immunology Section | LMI, NIAID |
| Others: | G. Caldes | Chemist | LMI, NIAID |
| | P. W. Stashak | Microbiologist | LMI, NIAID |
| | M. Fauntleroy | Biologist | LMI, NIAID |

COOPERATING UNITS (if any)

The Jackson Laboratory, Bar Harbor, ME (D. W. Bailey)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 0.3 | 0.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

B6.C congenic strains of mice, possessing chromosomal segments from high-responding BALB/cByJ (C) mice on the genetic background of low-responding C57BL/6ByJ (B6) mice, were examined for their ability to make an antibody response to Type III pneumococcal polysaccharide (SSS-III) and the alpha 1→3 determinant of bacterial (Leuconostoc) dextran B-1355. The results obtained affirmed that genes making a positive contribution to responsiveness to SSS-III are located on different chromosomes, i.e., chromosomes 1,3,4,5, and 9. At least one other gene also influences responsiveness to this antigen; it is closely linked to the H-17 locus which has not yet been assigned to a specific chromosome. Genes on chromosomes 1,4, and 5 were found to influence the magnitude of the antibody response to dextran B-1355. Some of these genes appear to be antigen-specific in their mode of action; however, others are not since they exert an influence on the antibody response to both SSS-III and dextran B-1355.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00144-21 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the antibody response to microbial polysaccharide antigens.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | P. J. Baker | Hd., Microbiology and Immunology Section | LMI, NIAID |
| Others: | J. Domer | IPA* | |
| | K. Elkins | Staff Fellow | LMI, NIAID |
| | P. W. Stashak | Microbiologist | LMI, NIAID |
| | M. Fauntleroy | Biologist | LMI, NIAID |

COOPERATING UNITS (if any)

*Department of Microbiology, Tulane University

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Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.3 | 0.7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mannans can produce either stimulation or suppression of the antibody response to Type III pneumococcal polysaccharide (SSS-III) and sheep erythrocytes (SRBC); the effect produced depends upon the concentration, molecular size, and chemical composition of the preparation of mannan used. These immuno modulatory effects appear to be T cell dependent since they are not demonstrable in athymic nude mice and the mannans used do not induce the non-specific proliferation of B lymphocytes.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00145-18 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Mode of action of thymus-derived (T) suppressor and amplifier cells.

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | P. J. Baker | Head, Microbiology & Immunology Section | LMI, NIAID |
| Others: | B. Prescott | * | |
| | C. E. Taylor | ** | |
| | P. W. Stashak | Microbiologist | LMI, NIAID |
| | M. Fauntleroy | Biologist | LMI, NIAID |
| | G. Caldes | Chemist | LMI, NIAID |

COOPERATING UNITS (*if any*)

*Biomedical Research Institute, Rockville, MD 20852; **Department of Microbiology & Immunology, Medical College of Pennsylvania, Philadelphia, PA 19129

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SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 0.3 | 0.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The transfer of bone marrow-derived precursors of antibody-forming cells (B cells) from mice immunized with Type III pneumococcal polysaccharide (SSS-III) results in the activation of suppressor and amplifier T cells that control the magnitude of the antibody response in recipient mice, immunized subsequently with SSS-III. Prior treatment of transferred B cells with an excess of an enzyme (polysaccharide depolymerase) capable of hydrolyzing SSS-III, does not alter the capacity of these cells to activate regulatory T cells. These findings indicate that the activation of regulatory T cells by immune B cells is not mediated by residual antigen on the surface of transferred cells.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00146-12 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Immunological Studies of Components Isolated from Bacteria, Parasites and Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | G. Caldes | Chemist | LMI, NIAID |
| Others: | P. J. Baker | Hd., Microbiology and Immunology Section | LMI, NIAID |
| | B. Prescott* | | |
| | P. W. Stashak | Microbiologist | LMI, NIAID |

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.6 | 0.1 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cellobiuronic acid, the basic structural unit of Type III pneumococal polysaccharide, was synthesized from octaacetyl cellobiose through the intermediate compounds, heptaacetyl bromocellobiose, heptacetyl benzylcellobiose, and benzyl cellobiuronic acid. Preliminary experiments were carried out towards the enzymatic synthesis of Type III polysaccharide by polymerization of the cellobiuronic acid through treatment with an enzyme derived from plants.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00153-08 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In vitro responses of human peripheral leukocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI : | Hsiao-Kun Chu | Visiting Fellow | LMI, NIAID |
| Others: | R. Asofsky | Chief, Laboratory of Microbial Immunity | LMI, NIAID |

COOPERATING UNITS (if any)

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 1.2 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


S11-B is a permanent line of human lymphocytes transformed in vitro with E-B virus (by S. Shaw, IB, NCI). ACF-9, a subclone of this line, express mIgM, mIgA, HLA, and HLA-DR antigens, and FcR(Hu). This line responds to a lymphokine(s) in human MLR culture fluids with (a) slowed growth, or death at high concentrations, and (b) with a concomitant tenfold increase in HLA-DR antigens, as judged by flow cytometry. There was no change in expression of HLA antigens, FcR, IgA or IgM in induced cells. The MLR culture fluids produced the change in HLA-DR at high dilution (e.g. $5\times10^{-2}$). Higher concentrations were needed to slow cell growth (work with J. Ambrus, LIR, NIAID).

Two human-mouse hybridomas, produced by immunizing human cells in vitro with tetanus toxoid, then fusing the stimulated cells with a mouse myeloma cell were found to be double secretors: each hybridoma, and more than 30 subclones of each secreted both IgM and IgG in high titer. Every cell in each clone stained with fluorescent labeled antibodies to human IgG and IgM. Elisa tests showed that each clone made both human IgG and IgM antibody to tetanus toxoid. These hybrids are the first examples of "biclonal" lines in which each immunoglobulin has antibody activity to the same antigen. These lines may be useful in the study of the "isotype switch".




PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00186-12 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of autoimmunity in inbred strains of mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI : | T. M. Chused | Senior Investigator | LMI, NIAID |
| Others: | R. Lal | Visiting Fellow | LMI, NIAID |
| | K. McCoy | Guest Worker | LMI, NIAID |
| | L. Edison | Biologist | LMI, NIAID |
| | E. Brown | Biologist | LMI, NIAID |
| | L. Kendrick | Biologist | LMI, NIAID |

COOPERATING UNITS *(if any)*

P. J. Baker, LMI, NIAID; H. Cooper, NCI

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 0.5 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The genetic control and immunlogic mechanisms of autoimmune disease is being investigated in the New Zealand strains of mice, their $F_1$ hybrids, and recombinant inbred lines derived from them. We have found 1) that enlargement of Lyt-$2^+$ T cells is significantly associated with the titer of anti-erythrocyte autoantibody and degree of hemolytic anemia; 2) T cell suppression is defective in old NZB mice; 3) NZB, and particularly the (NZB x NZW) $F_1$ hybrid, has a major resistance to induction of tolerance in the T cell subpopulation; 4) several abnormalities of proteins synthesized by lymphocytes from NZB mice can be demonstrated by two-dimensional gel electrophoresis. One, 16 kd in size, is observed only in enlarged NZB B cells, and may be associated with their pathologic spontaneous activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00203-06 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Applications of flow cytometry in immunology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | T. M. Chused | Senior Investigator | LMI, NIAID |
| Others: | B. J. Fowlkes | Senior Investigator | LMI, NIAID |
| | L. Edison | Biologist | LMI, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 0.2 | 0.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Flow cytometry, supported by advanced multiparameter data analysis, is being applied to immunologic problems in the following areas: 1) analysis of lymphocyte subset alterations in patients with AIDS, AIDS-related complex, and other immunologic disorders, 2) regulation of membrane potential by lymphocytes and neutrophilis, and 3) murine T and B cell differentiation and activation.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00423-02 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factor mediated regulation of B cell growth and differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | M. Howard | Visiting Scientist | LMI, NIAID |
| Others: | P. Stein | Guest Worker | LMI, NIAID |
| | P. Dubois | Guest Worker | LMI, NIAID |
| | D. Ennist | Staff Fellow | LMI, NIAID |
| | G. Wesley | Medical Staff Fellow | LMI, NIAID |
| | D. Greenblatt | Medical Staff Fellow | LMI, NIAID |
| | T. Teranishi | Visiting Associate | LMI, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 4.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


B cell immune responses are regulated by a family of T cell and macrophage derived glycoproteins. Here we extend our understanding of this regulation by demonstrating the following:

(1) The proliferative response of unstimulated resting B cells to Tl-2 antigens shows an absolute requirement for BSF-1, can be augmented by IL-1, and suppressed by IFN-$\gamma$. In contrast Tl-1 antigens induce antigen-specific proliferation in the absence of exogenous B cell stimulatory factors.

(2) Continuously growing bone marrow cell lines which are predominantly pre-B cell in nature proliferate in response to T cell and macrophage derived factors. This proliferation can be suppressed by IFN-$\gamma$.

(3) Continuously growing Lyl-positive B cell lines and clones secrete interleukin-1.

(4) Lymphokines which enhance the proliferation of $BCL_1$ tumor cells show biochemical and functional heterogeneity.

(5) It is possible to produce cloned B cell hybrids which respond to BSF-1 by increased Ia expression, and which may therefore prove useful tools for analysis of BSF-1 receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00425-01 LMI

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lymphocyte physiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | T. M. Chused | Senior Investigator | LMI, NIAID |
| Others: | H. A. Wilson | Guest Worker | LMI, NIAID |
| | D. Greenblatt | Medical Staff Fellow | LMI, NIAID |
| | L. Edison | Biologist | LMI, NIAID |
| | E. Brown | Biologist | LMI, NIAID |

COOPERATING UNITS (if any)

B. Seligmann, LCI, NIAID; R. Tsien, Univ. California at Berkley

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.3 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The process of signal transduction across the lymphocyte plasma membrane is under investigation. Novel fluorescent probes of physiologic parameters such as membrane potential, intracellular pH and intracellular calcium, in conjunction with the high sensitivity and single cell resolution of flow cytometry, are being utilized.

We have found that positively charged cyanine dyes are not suitable for measuring membrane potential in cells containing mitochondria, but that negatively charged oxonol dyes are reliable indicators. Lymphocytes and monocytes, but not granulocytes, buffer membrane potential over more than the physiologic range of extracellular potassium ion. T lymphocytes, but not B lymphocytes, possess a calmodulin-dependent, calcium-sensitive potassium channel.

A new calcium probe, indo-1, is very useful for measuring intracellular free calcium ion. We find that blocking the IgG Fc receptor of B cells significantly prolongs the calcium transient induced by anti-immunoglobulin.



PHS 6040 (Rev. 1/84) GPO 904-917

LABORATORY OF MOLECULAR MICROBIOLOGY
1985 Annual Reports
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Public Health Service - National Instiutes of Health
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID
October 1, 1984 - September 30, 1985

Dr. Malcolm A. Martin, Chief

The laboratory of Molecular Microbiology (LMM) applies molecular biological techniques to study the structure and regulation of prokaryotic and eukaryotic genes. Although a major focus has been animal virus systems, bacteria and mycoplasmas are also investigated. Relying heavily on nucleic acid hybridization, restriction enzyme mapping, molecular cloning, DNA sequencing, in vitro mutogenesis, and DNA transfection, LMM staff have productively investigated a variety of genetic elements that play important roles in the interaction of microorganisms and their host cells. In many instances, newer technologies have been combined with more conventional virologic and bacterial assay systems particularly for evaluating novel and biologically active DNA recombinants.

LMM has many of the components of a university microbiology department. The Bacterial Virulence Section, headed by Dr. Donald LeBlanc and located in Building 550, Frederick Cancer Research Facility (FCRF) has examined a variety of streptococcal plasmids focusing primarily on the structure of their replicons and their dissemination in the ecosystem. A new program, focusing on the expression of antibiotic resistance plasmids in the anaerobic bacterium, Bacteriodes fragilis, is rapidly gathering momentum. The Mycoplasma Section, also located at FCRF, and headed by Dr. Joseph Tully, is concerned with the isolation, characterization, and classification of wall-free prokaryotes (mollicutes) particularly those associated with man. Members of the Viral Biology Section, located in Buildings 5 and 7 in Bethesda utilize adenovirus and murine retrovirus systems to assess cellular and viral determinants involved in productive and transforming infections. The Biochemical Virology Section, headed by Dr. Malcolm Martin, located in Building 5, Bethesda, investigates the molecular biology of mammalian retroviruses concentrating primarily on murine leukemia viruses and the acquired immunodeficiency syndrome (AIDS) retrovirus (RV).

During the past year several members of the Biochemical Virology Section initiated new research programs focusing on the structure and expression of the AIDS RV genome. This work was carried out as a collaborative effort with Drs. Tom Folks, Clifford Lane, and Anthony Fauci of the Laboratory of Immunoregulation, who provided expertise in cell biology and clinical immunology. An early product of this concerted effort has been the development of a continuous growing, IL-2 independent human T lymphocyte line for the efficient propagation of the AIDS RV. This cell line (A3.01) was >95% Leu-3+ and exhibited the characteristic cytopathic changes associated with virus infection of PHA stimulated human lymphocytes including cell death. Molecular clones of the AIDS proviral DNA were isolated from a human lamdaphage gene library contructed from infected A3.01 cells. Subgenomic segments of the AIDS RV genome were then used in a variety of studies

including the detection of viral nucleic acids in cells obtained from AIDS patients. Our results indicate that conventional dot, slot, and Northern blot hybridization procedures are not sensitive enough to detect the rare cell from a patient that expresses AIDS RV mRNA. We have turned instead to in situ hybridization techniques for the identification of viral nucleic acids in clinical specimens.

Three other accomplishments involving the molecular biology of the AIDS virus deserve mention. Detailed restriction maps of six North American and three Zairian isolates were prepared during the course of a study evaluating the genomic stability of the AIDS RV. The results of this analysis conclusively demonstrate extensive restriction enzyme polymorphisms among the different isolates. Changes were concentrated to the 3' half of the viral genome affecting primarily the envelope coding sequence. No geographical specificity was apparant among the North American isolates. Northern blot analysis revealed several surprises concerning AIDS RV mRNAs. Unlike other replication-competent mammalian retroviruses which express only two discrete viral RNA species (full-length genomic/gag-pol and 3.0 kb env mRNA), $T4^+$ lymphocytes undergoing productive infection with the AIDS virus contain five size-classes of RNA: 9.1 kb (genome/gag-pol mRNA) 5.5 kb, 5.0 kb, 4.3 kb (env mRNA) and 1.8-2.0 kb. Using subgenomic probes and labeled synthetic oligonucleotides, the 5.5 and 5.0 kb RNAs have been mapped to the unique "A" open reading frame (located been the pol and env genes) and the 1.8-2.0 kb species to an open reading frame located 3' to env coding sequences. Another important achievement has been the construction of an infectious AIDS RV DNA clone that generates biologically active progeny virus particles following its introduction into sensitive human cells. Site specific mutagenesis is presently being utilized to inactivate different viral genes as part of a functional evaluation of the viral genome.

Considerable progress has been made in our understanding of endogenous murine leukemia virus (MuLV) sequences and their relationship to recombinant murine retroviruses isolated from leukemic tissue. Labeled DNA probes, specific for the mink cell focus (MCF) forming or xenotropic MuLV envelope genes were used for the detection of the corresponding provirus in mouse chromosomal DNA as well as of specific mRNAs in different mouse tissues. The results of Southern blot analyses using these labeled DNAs indicate that inbred mice contain many more copies of proviruses with an associated MCF envelope than endogenous proviral DNAs with a xenotropic env gene. A similar investigation of feral mice indicates that many harbor neither type of endogenous provirus suggesting that in contrast to inbred animals, wild mice evolved from a lineage that escaped exogenous infection with an ancestral MuLV-related retrovirus. The MCF MuLV env probe was employed in Northern blot analyses to analyze the $polyA^+$ RNA present in five different organs of AKR/N mice ranging in age from 5 weeks to 6 months. This is a "high" leukemia incidence strain of inbred mice that spontaneously develops thymic lymphomas/leukemias containing recombinant MCF MuLVs. MCF env specific 7.2 and 1.8 kb mRNAs were detected in thymus tissue (and no other organs) in 5 week old animals. By 3 months, a novel, full-length 8.2 kb MCF env specific RNA species, restricted to the thymus, was also detected, presumably representing the recombinant MCF MuLV genome. A cDNA

corresponding to the 7.2 kb mRNA, the putative precursor to the 8.2 kb MCF MuLV genome, has been molecularly cloned and is presently being analyzed.

Members of the Bacterial Virulence Section continued utilizing molecular and genetic techniques to study bacterial plasmids and plasmid-mediated functions. In a series of experiments designed to assess the extent to which streptococcal antibiotic resistance genes have been disseminated in the natural environment, it was shown that the genetic determinants encoding resistance to streptomycin, kanamycin and erythromycin, originally found on the extensively studied *S. faecalis* plasmid pJH1, were also present on novel plasmids in nearly 70% of multiple drug resistant human and animal isolates of group D streptococci. A novel spectinomycin resistance gene was cloned from plasmid DNA carried by a human clinical isolate of *S. faecalis*. Additional studies indicated that this determinant was present in *S. faecalis* isolates of animal origin prior to its emergence in the human strain. Recent clinical isolates of the human pathogen, *Mycoplasma hominis*, have been shown to be highly resistant to tetracycline, the antibiotic of choice in the treatment of *Mycoplasma* infections. In collaboration with Dr. Marilyn Roberts and colleagues, University of Washington, Seattle, it was shown that this newly acquired resistance trait was due to the presence of the streptococcal tetracycline resistance determinant, *tet*M. These results constituted the first evidence for the presence of *tet*M in an unrelated genus and suggested the spread of *tet*M from *Streptococcus* to *Mycoplasma*. Considerable progress has also been made in studies of antibiotic resistance plasmids in the anaerobic bacterium, *Bacteroides fragilis*. Transposon-like structures encoding clindamycin resistance on three different *Bacteroides* R plasmids were cloned in *E. coli*. All three structures were shown to be bounded by a homologous 1.2 kb directly repeated sequence (DRS). The transposon-like elements from two of the plasmids shared more than 90% DNA sequence homology, while the third element had diverged significantly. In addition to the DRS, only a 0.85 kb DNA segment, identified as the clindamycin resistance gene of this latter structure, was homologous to the two other transposon-like elements. A genetic transformation procedure and gene cloning system was developed for *B. fragilis*. This system with newly constructed *Bacteroides* vectors and *Bacteroides*-*E. coli* shuttle vectors, is currently being used to examine the structure and expression of *Bacteroides* antibiotic resistance genes.

## Specific Research Accomplishments

*A comparative analysis of multiple antibiotic resistance plasmids from group D streptococci of human and animal origin*. Cloned restriction endonuclease fragments containing the streptomycin (Sm), kanamycin (Km) or erythromycin (Em) resistance genes from the *Streptococcus faecalis* plasmid pJH1 were used as probes in hybridization reactions containing plasmid-enriched DNA from 91 group D streptococcal isolates resistant to Sm, Km and Em. Nearly 70% of the strains examined contained DNA that hybridized to each of the cloned fragments from pJH1. Forty-six strains were from healthy chickens on farms in four states and the Delmarva Penisula; 21 were from healthy pigs in six states; 24 were human clinical isolates from the District of Columbia,

Texas, Thailand, and Chile. Five plasmids were examined in more detail. Three were from human isolates obtained in London, Ann Arbor, and Washington, D.C.; two were from pigs on farms in Illinois and Nebraska. Although these plasmids were quite different in size, each contained the Sm, Km and Em resistance determinants on a single 13-20 kb EcoRI fragment. Southern blot hybridizations and additional restriction endonuclease digests revealed extensive homology and virtually identical restriction maps within a 9-11 kb region of each plasmid which included the coding sequence for the resistance determinants. (LeBlanc, Lee and Inamine)

Characterization of a novel spectinomycin resistance determinant in Streptococcus faecalis. A human clinical isolate of Streptococcus faecalis, resistant to high levels (>2000 µg/ml) of spectinomycin (Sp), transferred this resistance to a plasmid-free recipient strain. Although other gram-positive and gram-negative bacterial strains resistant to high levels of Sp have also been resistant to streptomycin (Sm), the plasmid from this strain appeared to mediate resistance to Sp only. Cell-free extracts of a Sp-resistant transconjugant contained adenylylating activity for Sp, but not Sm, as opposed to the Sp modifying activity associated with other resistant bacterial isolates. A 1.1 kb ClaI/NdeI fragment was cloned from the plasmid DNA purified from the transconjugant. Streptococcus sanguis and E. coli transformants, carrying this fragment on appropriate vectors were able to express high levels of Sp resistance. Cloned fragments were used as hybridization probes to identify, in four Sp-resistant group D streptococci isolated from animal sources, an 8 kb segment of DNA present in the Sp-resistant transconjugant. Preliminary data indicated that this Sp resistance determinant was present in animal strains prior to its emergence in the human S. faecalis strain. (Inamine, Lee and LeBlanc)

Dissemination of a streptococcal tetracycline-resistant determinant among strains of Mycoplasma hominis. Mycoplasms species have been considered universally susceptible to tetracycline (Tc), but in the past several years clinical isolates have been described with increased resistance to this antibiotic. Several of these latter strains were shown to contain DNA sequences homologous to the streptococcal Tc resistance determinant, tetM. None of the susceptible strains tested contained DNA homologous to the Tc-specific probe. These results constituted the first evidence for the presence of tetM in an unrelated genus and suggested the spread of tetM from Streptococcus to Mycoplasma. (LeBlanc)

Plasmid-mediated lactose metabolism in group N streptococci. The ability of group N streptococci to metabolize lactose is plasmid-mediated. Two genetic determinants associated with this trait, encoding phospho-β-galactosidase (p-gal) activity and a lactose-specific component of the PEP-dependent phosphotransferase system (lac-PTS), were cloned in E. coli using the pUC19 vector and a lactose plasmid from a strain of Streptococcus cremoris. The p-gal activity was expressed in E. coli. as determined by a microtiter assay employing ONPG-6-phosphate as a substrate. The lac-PTS activity could not be detected in the E. coli transformants. A cloned 3.5 kb PstI/AvaI fragment transformed mutants of Streptococcus sanguis

deficient in lac-PTS and p-gal activities (lac83), or only p-gal (lac8), to a lactose positive phenotype. A 2.6 kb SstI/AvaI fragment transformed the lac8, but not the lac83, mutant. Southern blot hybridizations confirmed the presence of plasmid-specific sequences in the S. sanguis transformants, identified a fragment of S. sanguis chromosomal DNA into which the lactose-specific determinants integrated, and established the presence of identical genetic determinants for lactose metabolism in plasmids from S. cremoris and streptococcus lactis. (Inamine, Lee and LeBlanc)

Genetic and molecular comparisons of antibiotic resistance plasmids in anaerobic bacteria of the genus Bacteroides. A comparison of transposon-like structures encoding clindamycin (Cc) resistance in three Bacteroides R-plasmids revealed that each was bounded by the same 1.2 kb direct repeat sequence. These transposon-like structures varied in size from 5.2-8.4 kb and two of these (from pBF4 and pBFTM10) shared more than 90% homology. pBI136 was the largest plasmid of the three and had diverged significantly in structure; only the direct repeat sequence and a 0.85 kb region assigned to the Cc resistance gene of the pBI136 transposon-like element had homology to the other two plasmids. The putative Cc resistance genes in both pBF4 and pBO136 is situated adjacent to a copy of the direct repeat sequence but are in opposite orientations and abutted to opposite copies of the direct repeat sequence. (Smith)

Development of gene cloning systems for the anaerobe Bacteroides fragilis. The inability to attain expression of Bacteroides antibiotic resistance determinants in E. coli has hampered understanding of genetic organization in Bacteroides R-plasmids. In order to overcome this problem a cloning system was designed for B. fragilis; these organisms are refractory to commonly used transformation procedures so a novel polyethylene glycol transformation method was developed. Plasmid cloning vectors able to replicate in Bacteroides or in Bacteroides and E. coli were constructed and their genetic and structural properties determined. Construction and utilization of these chimeric plasmids provided genetic evidence for the location of the Cc resistance gene in the three Bacteroides R-plasmids. (Smith)

Biological and molecular features of Acholeplasmas. Sterol non-requiring, wall-free mycoplasmas (acholeplasmas) recovered from human and insect/plant sources were characterized. Acholeplasma oculi and A. laidlawii strains were identified, for the first time, from human amniotic fluid and fetal tissues, respectively. Other isolates of non-human origin were serologically distinct from known Acholeplasma species. These strains demonstrated the biological and molecular features of acholeplasmas, including a DNA genome size of about 1 x $10^9$ daltons, a G+C DNA content of 30-35 mole %, and growth in the absence of added sterol. (Tully)

Attachment moiety in mycoplasma genitalium. A specific 143 K protein has been identified as the possible component in the adherence of this mycoplasma to tissue cells. This protein is distinct from the 165 K protein (P1) identified as the principal attachment moiety in Mycoplasma pneumoniae, another pathogenic mycoplasma for man. Antibody to the 143 K protein also appears in the serum of chimpanzees

given an experimental genital tract challenge with Mycoplasma genitalium. These findings are similar to our earlier observation that antibody to P1 protein occurs in humans convalescing from respiratory infection with M. pneumoniae. (Tully)

Expression of histocompatibility antigens on the surfaces of DNA virus transformed cells. Adenovirus 12 (Ad12) is considered to be a highly oncogenic virus because this virus induces tumors when injected into hamsters and mice and can transform normal rodent cells in tissue culture to cells that produce tumors when injected into immunocompetent animals. Ad2 and Ad5 are classified as non-oncogenic viruses since they fail to induce tumors when injected into hamsters and mice. Both Ad2 and Ad5 can transform normal rodent cells in tissue culture to neoplastic cells that produce tumors only in immunocompetent animals. Data have been published suggesting that the E1A region of the Ad12 genome, but not the Ad5 genome, blocks the expression of Class I histocompatibility antigens on the surfaces of transformed rodent and mouse cells. Like Ad12, SV40 and polyoma are also highly oncogenic for Syrian hamsters; furthermore, SV40 and polyoma transformed hamster cells have the additional capacity to produce tumors with almost equal efficiency in both syngeneic and allogenic inbred hamsters--a property that could be explained by alterations in the expession of cell surface Class I histocompatibility antigens. As there are no specific reagents available that detect hamster Class I antigens, these cells are being studied for the expression of β2 microglobulin, a protein that is known to be closely associated with Class I proteins on cell surfaces. Thus far, no major differences have been detected in the expression of β2 microglobulin Ad2, Ad5 or Ad12 transformed hamster cells and SV40 and polyoma transformed cells seem to express an increased amount of this protein compared to adenovirus transformed cells or normal cells. If the presence of β2 microglobulin on hamster cells is a reflection of the presence of Class I antigens, then these results suggest that altered expression of Class I is not likely to be a factor in determining the tumor inducing capacity of DNA virus transformed hamster cells. (Haddada, Lewis)

Adenovirus 2/5 E1A Gene Induction of Cytolytic Susceptibility in Transformed and Infected Cells. Previous studies suggested that viral oncogenes integrated into neoplastic cell DNA may cause increased susceptibility of cells to lysis by NK cells and activated macrophages. Certain transforming DNA viruses (e.g., non-oncogenic adenovirus (Ad2 and Ad5) induce increased cytolytic susceptibility during transformation. A series of hamster, rat and human cells transformed by overlapping viral gene segments has been studied to define the Ad2 and Ad5 viral gene regions that control this neoplastic cell phenotype. The results obtained indicate that the intact function of the Ad2 or Ad5 E1A proteins increase cellular cytolytic susceptibility indirectly by inducing the expression of an otherwise quiescent cell genotype that remains to be defined. We speculate that differences in the functions of viral or non-viral E1A-like transforming genes may account for differences in neoplastic cell susceptibility to destruction by immunologically non-specific host cellular defenses. (Cook, Walker, Lewis)

Genetic mapping of chromosomal genes involved in viral oncogenesis. Hamster X mouse somatic cell hybrids and genetic crosses were analyzed to chromosomally localize proviral genes, cellular oncogenes, and tumor-specific integration sites. One provirus, representing a putative precursor of the MCF MuLV, was mapped to chromosome 11. A mouse mammary tumor (MMTV) provirus of BALB/c was mapped to chromosome 6 and was shown to be similar to the other two endogenous BALB/c MMTV proviral DNAs located near immunoglobulin genes. Another MMTV provirus in C3H/HeJ was mapped to chromosome 14. The cellular homologues of the Rel and Erbb oncogenes were mapped to chromosome 11, and it was shown that sequences homologous to the 3' and 5' ends of v-ets are on two different mouse chromosomes. Two tumor-specific integration sites have now been mapped to chromosome 15 (Mis-1) and chromosome 17 (Int-3). (Kozak, Khan, Prakash, Callahan, O'Brien, Silver, Jolicoeur)

Characterization of wild mice for sensitivity to MuLVs. Many wild mice lack the Fv-1-type restriction of MuLVs characteristic of inbred animals. These mice are equally susceptible to N- and B-tropic viruses. Genetic crosses with M. spretus and M. m. praetextus revealed that these wild mice carry a novel nonrestrictive allele at Fv-1. Additional experiments demonstrated that wild mouse populations differ from laboratory mice in their in vitro sensitivity to infection by exogenous xenotropic virus. This sensitivity is governed by a single dominant locus on chromosome 1 termed Sxv. This gene appears to represent a wild mouse polymorphism of the MCF MuLV receptor determinant. (Kozak).

Molecular mechanisms of leukemogenesis by Friend MuLV. In analyzing proviral insertions in Friend (F) murine leukemia virus (MuLV) induced tumors, a common proviral integration region for this retrovirus in ~10% of lymphoid and myeloid leukemias was discovered. This integration region maps to mouse chromosome 7 very near a common integration region (Int-2) for mouse mammary tumor virus in mammary carcinomas. This integration region may represent a new oncogene or, alternatively, could indicate that the Int-2 locus is involved in lymphoid and myeloid leukemias as well as mammary carcinoma. The human equivalent of this integration site is presently being cloned and rearrangements or abnormal expression of this locus in human malignancies are being evaluated. (Silver)

New DNA probes specific for MCF and xenotropic MuLV env sequences have been developed. DNA segments (100 bp) from analogous portions of the MCF and xenotropic gp70 env coding regions were isolated and subcloned into a M13 phage vector. No cross-reactivity could be demonstrated in Southern blot hybridizations of cloned xenotropic or MCF proviral DNAs. The labeled env-specific DNAs did not react with ecotropic or amphotropic MuLV sequences. We have previously shown that multiple copies of MuLV proviral DNA are present in mouse chromosomal DNA. An analysis of DNA prepared from a broad range of inbred mice indicated that many more endogenous proviruses with a MCF env than ones with a xenotropic MuLV env segment exist in the mouse genome. Wild mouse strains were more diverse. Some had neither MCF nor xenotropic MuLV-reactive sequences, some contained MCF env sequences only and others contained both. (O'Neill, Repaske, Khan, Hoggan, Kozak)

Expression of MCF related mRNA in AKR mice. Expression of RNA transcripts containing MCF env-reactive sequences was monitored in a variety of AKR mouse tissues ranging in age from 5 weeks to 6 months using a MCF MuLV env-specific probe developed during the past year in our laboratory. MCF env RNA transcripts, 1.8 and 7.2 kb in size, were detected in the thymus of 5 week old animals. At 3 months, prior to the appearance of gross thymomas, full-length MCF MuLV genomes could be identified in preparations of polyadenylated thymus RNA. Using a different MuLV env specific probe, ecotropic MuLV mRNA transcripts (8.2 kb) could be detected in all tissues and increased in amount with the age in the AKR mice examined. These data suggest the recombinational event giving use to MCF viruses in the thymus are related to the appearance of the 1.8 and/or 7.2 kb transcripts and the recombination(s) are accomplished by 3 months of age. (Laigret, Khan, Rabson, Boulukos, Repaske)

The LTR and 3' pol regions of leukemogenic and non-leukemogenic MCF MuLVs contain significant nucleotide differences. Infectious molecular cloned DNAs were obtained of leukemogenic MCF-13 and non-leukemogenic MCF-111A MuLVs. The nucleotide sequence of the LKTRs, 3' pol and env regions was determined. The results of comparative sequence analysis indicated: the LTR associated with MCF-13 is closely related to that present in xenotropic MuLVs, whereas the LTR sequence of MCF-111A is identical except for 1 bp to the ecotropic proviral LTR; no significant sequence divergence was seen between MCF-13 and MCF-111A in the env region; a 12 bp nucleotide stretch, characteristic of leukemogenic MCF MuLVs, was conserved in MCF-13 in the 3' pol region but was lacking in MCF-111A. These results suggest that the leukemogenic potential of MCF-13 may reside in LTR and 3' pol sequences. (Theodore and Khan)

A new transforming mouse retrovirus contains the ras oncogene. Two independent isolates of a transforming retrovirus was isolated from splenic tumors of NFS mice following inoculation with C25 LI MCF virus, originally identified by Dr. Janet Hartley, LIP, NIAID. A biologically active molecular clone of the transforming virus was extensively characterized. The 8.8 kb viral genome contained gag and pol genes indistinguishable from ecotropic MuLV based upon its restriction enzyme cleavage map. Approximately 1000 bp of late pol and env genes were replaced with non-viral sequences identified as ras by hybridization. Nucleotide sequencing of this region showed a 567 bp segment encoding p21 ras that was flanked on both 5' and 3' ends by presumptive mouse sequences. Oncogenicity of p21 coding sequences is based upon the arginine substitution at amino acid 12. The ras sequence was closely related to bas, H-ras, and Rasheed-ras, but more distantly related to T24 ras. (Frederickson, Rutledge, O'Neill, Theodore, Martin and Hartley)

The $tRNA^{Glu}$ class of endogenous retroviral sequences is present in the primate germline extending from humans back through old world monkeys. A unique feature of the full-length class of human endogenous retroviral sequences is the presence of a primer binding site (pbs) complementary to $tRNA^{Glu}$. No other known infectious or endogenous mammalian retrovirus has such a pbs. The env segment of the full-length human endogenous retroviral family failed to

cross-hybridize or to have significant polynucleotide sequence homology with known infectious retroviruses. On the other hand, a labeled human retroviral env probe, hybridized quite well to chimpanzee, orangutan, gibbon, baboon and African green monkey chromosomal DNAs; it failed to react with owl monkey, squirrel monkey, galago and rodent DNA. An African green monkey (AGM) gene library was screened with a human endogenous retroviral env probe and representative regions of clones obtained were sequenced. Nucleotide sequence homology of the endogenous AGM and a human retroviral clone ranged from 80-88%; the primer binding site associated with the AGM proviral DNA was also complementary to $tRNA^{Glu}$. This family of endogenous mammalian retrovirus thus entered the primate germline sometime after old world and new world monkeys diverged (about 30-60 million years ago). (Martin, Repaske, Rabson).

Some human endogenous retroviral segments have undergone amplification. The number of primary germline integrations of human and endogenous retroviral sequences is far less than would be suggested by the 35-50 copies currently present in human chromosomal DNA. Many of these retroviral structures appear to have arisen from amplification of large DNA segments that would include both retroviral flanking cellular sequences. Cellular DNA, located immudiately 3' to viral sequences present in one of the human retroviral clones, annealed to obviously recruited BamHI and HindIII junction fragments with sizes predicted from existing clones. Southern blot analysis of chimpanzee and African green monkey DNAs revealed the presence of similar recruited bands in the former but not the latter. This result indicates that the proposed amplification event, originally detected in human DNA, occurred prior to the divergence of homomids from the common ancestor with African apes. Additional experiments, employing a panel of rodent X human somatic cell hybrids, demonstrated that the 35-50 copies of retroviral sequences associated with the human genome have been dispersed to multiple human chromosomes. (Martin, Steele, O'Brien).

Molecular cloning of the AIDS RV. A molecular clone of the integrated LAV provirus was obtained by screening a lambdaphage library of virus-infected cellular DNA with a $^{32}P$-labeled cDNA probe. To prepare this probe, LAV virus was first purified by rate-zonal centrifugation from 14 liters of infected A3.01 cells and the RNA, isolated from banded virus, was labeled in vitro by reverse transcription. A clone consisting of 8 kb of flanking cellular DNA and the 5' 8.4 kb of the AIDS RV was isolated from the lambdaphage library and subcloned into various plasmid vectors. Low and moderate stringency hybridization experiments indicated: 1) the AIDS RV failed to hybridize to molecular clones of HTLV-I or HTLV-II proviral DNAs, and 2) exhibited no cross-reactivity with cloned endogenous human retroviral segments. (Benn, Theodore and Martin)

Identification of RNA transcripts from the novel open reading frames of the AIDS Retrovirus. The expression of AIDS RV-specific RNA in cytolytically infected cells has been studied by Northern blot hybridization. Five major species of AIDS RV transcripts have been identified: 1) a 9.1 kb RNA including full-length viral transcripts and putative gag-pol mRNA; 2) a 5.5 kb RNA that hybridizes to LTR, "A," env, and "B" region probes; 3) a 5.0 kb RNA hybridizing to LTR, "A," env, and "B" region DNA; 4) a 4.3 kb RNA that hybridizes to LTR, env, and "B" regions, the putative spliced env mRNA; and (5) a family of 1.8-2.0 kb RNAs that hybridize to LTR and "B" region probes. A similar analysis of H9 cells, a continuous human T lymphocyte line that chronically produces the AIDS RV and is not killed by the virus, indicated that the same five mRNAs are synthesized. cDNA clones of these viral RNA are currently being isolated and their patterns of synthesis analyzed. (Rabson, Daugherty, Martin)

Genomic heterogeneity of AIDS RV isolates from North America and Zaire. Restriction enzymes, known to cleave the AIDS RV provirus, were used for the construction of detailed restriction maps of five New York, a single Alabama and three Zairian viral DNAs. For this analysis, contiguous subgenomic segments of a cloned LAV provirus were labeled in vitro and used to probe Southern blots of infected cellular DNA. The results obtained indicate: (1) all AIDS RV isolates contain several conserved "signature" restriction sites; (2) with the exception of LAV and HTLV-III, the North American isolates were all different from one another and exhibited no geographical specificity; (3) the African isolates as a group were significantly more diverse than those from North America; and (4) the genomic variability in the various isolates is concentrated within the env gene. (Benn, Folks, Martin)

## Administrative, Organizational and Other Changes

During the past year several members of Laboratory of Viral Diseases were administratively transferred to LMM including Drs. Christine Kozak, M. David Hoggan, and Hidetoshi Ikeda and their support staff. Dr. Eugenio Santos, who received postdoctoral training in Dr. Mariano Barbacid's laboratory, joined LMM as an independent investigator to study the structure and function of cellular oncogenes. During the past year Drs. Julia Inamine and Steven Benn completed postdoctoral training appointments; in May, 1985 Dr. Howard Gendelman joined LMM as an Expert Consultant. In August, 1985 Mrs. Joan Barnhart retired from NIH after 21 years of government service.

## Honors and Awards

Malcolm Martin

Invited speaker: NIAID Workshop, "Animal Models of Retrovirus Infection," Hamilton, Montana; November, 1984.

Invited discussant: Fogarty International Center Conference, "Oncogenes, Cell Growth, and Cancer," Bethesda, Maryland; November, 1984.

Invited speaker: European Community Workshop, "Viral Etiology of AIDS," Paris, France; December, 1984.

Invited speaker: Albert Einstein School of Medicine, New York, New York; January, 1985.

Organizer and invited speaker: Wallace Rowe Symposium on Animal Viruses, Bethesda, Maryland; February, 1985.

Organizer and invited speaker: EPA Conference, "Genetically Altered Viruses and the Environment." Cold Spring Harbor, New York; April, 1985.

Invited speaker: AAAS Annual Meeting, "Environmental Aspects of Genetically Altered Viruses," Los Angeles, California; May, 1985.

Invited speaker: Gordon Research Conference on Animal Cells and Viruses, Tilton, New Hampshire; June, 1985.

Organizer and symposium speaker: American Society for Virology, annual meeting, "Immunosuppressive Viral Infections," Albuquerque, New Mexico; July, 1985.

Invited speaker: Cold Spring Harbor Conference, "Modern Approaches to Vaccines," Cold Spring Harbor, New York; September, 1985.

Fellowship Review Panel, Life Sciences Research Foundation, Baltimore, Maryland.

Donald LeBlanc

Invited speaker: Department of Microbiology, University of Massachusetts, Amherst, Massachusetts; April, 1985.

Invited speaker: Annual convention, American Veterinary Medical Association, Las Vegas, Nevada; August, 1985.

Consultant, Program Advisory Group, FDA, Center for Veterinary Medicine.

Arnold Rabson

Invited speaker: Dana Farber Cencer Center, Harvard Medical School, Boston, Massachusetts; October, 1984.

Invited speaker: Departments of Pathology and Microbiology, Case-Western Reserve Medical School, Cleveland, Ohio; April, 1985.

Invited speaker: Department of Biochemistry, Brown University Program in Medicine, Providence, Rhode Island; May, 1985.

C. Jeffrey Smith

Invited speaker: Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia; February, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00011-20 LMM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 cherecters or less. Title must fit on one line between the borders.)

Studies of Small DNA Containing Viruses Belonging to the Family Parvoviridae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


PI: M. David Hoggan Senior Scientist LMM, NIAID


COOPERATING UNITS (if eny)


William L. Mengling — National Animal Disease Center, Ames, Iowa

Ronald K. Wilder — A&R, NIADDKD


LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.6 | 0.1 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


A parvovirus has been reportedly isolated from synoviral fluid of rheumatoid arthritis patients. All attempts to identify sequences related to 9 different known parvoviruses in diseased synoviral tissues, using a standard DNA hybridization dot blot test, have been negative.

Porcine parvovirus infection continues to be the primary cause of infertility in sows in the U.S.A. and elsewhere. Using high titered polyclonal antibody and purified antigens prepared from different parvoviruses; a highly sensitive and specific test for procine parvoviruses antibody using the ELISA assay is being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00013-22 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunobiology and Pathogenesis of DNA Virus Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Andrew M. Lewis, Jr. | Senior Investigator | LMM, NIAID |
| Others: | John A. Sogn | Senior Investigator | LIG, NIAID |
| | Henrietta Kulaga | Staff Fellow | LIG, NIAID |
| | Hedi Haddada | Visiting Fellow | LMM, NIAID |

COOPERATING UNITS *(if any)*
Jim Cook — National Jewish Hospital and Research Center, Denver, CO

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.0 | 1.0 | 4.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our recent studies of DNA virus infected cells suggest that the early genes of DNA viruses impart specific levels of susceptibility or resistance to cellular immune rejection to these cells. In DNA virus transformed cells, the susceptibility-resistance phenotype may determine the tumor inducing capacity of those cells. In in vitro, assays the susceptibility-resistance phenotype can be reflected in the ability of infected or transformed cells to be lysed by natural killer cells or activated macrophages. At this junction, the induction of the susceptibility phenotype-as expressed by hamster cells infected or transformed by adenovirus (Ad2)-appears to govern the phenotype of the affected cell; whereas, the expression of the resistant phenotype may be due to a species specific inability of certain viruses to induce susceptibility in normal cells which are inherently resistant. In support of this concept, we have found a dose dependent correlation between the level of Ad2 early gene products expressed in transformed hamster cells and: 1) their level of susceptibility to NK cell lysis, 2) their expression of the cytoskeletal proteins - actin and fibronectin, 3) their cytomorphology. We have also developed data which suggests that the induction of susceptibility is associated with the expression of the 13S mRNA of the E1A region of the Ad2 and Ad5 genomes. As rodent cells can be immortalized by the same segment the cDNA 5' terminus of 13S of the Ad2 and Ad5 genomes that fail to induce susceptibility and as this region of the genome is not known to induce virus-specific cell surface proteins, cell immortalization towards neoplasia is an event independent of the induction of susceptibility to lysis by immunologically non-specific host effector cells. In addition, susceptibility to lysis by effector cells may not be associated with the presence of virus-specific proteins on the surface of susceptible cells.

COOPERATING UNITS (Cont'd.)

| | | |
|---|---|---|
| Arthur S. Levine | Scientific Director | ODS, NICHD |
| Cephas T. Patch | Senior Investigator | OSD, NICHD |
| Kimihiro Akagi | Visiting Fellow | OSD, NICHD |
| Joseph B. Bolen | Senior Staff Fellow | PB, DCT, NCI |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00027-18 LMM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Joseph G. Tully | Chief, Mycoplasma Section | LMM, NIAID |
| Others: | David L. Rose | Research Microbiologist | LMM, NIAID |

COOPERATING UNITS (if any)

G. Cassell, Univ. of Alabama, Birmingham, AL
R. Whitcomb, USDA, Beltsville, MD
J. Baseman, Univ. of Texas, San Antonio, TX
A. Buck, Univ. of Texas, Galveston, TX
J. Bove, Univ. of Bordeaux, France

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Mycoplasma Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.0 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These efforts cover both basic and applied aspects of mycoplasmas and related wall-free prokaryotes (mollicutes), including their molecular biology, membrane and cellular components involved in attachment, virulence, or immunological interrelationships, and their possible role in human disease or diseases of uncertain etiology. Current efforts involve the characterization of a group of sterol non-requiring mollicutes (genus *Acholeplasma*) isolated from variety of human tissue sites and from other environmental origins. A strain of *Acholeplasma oculi* has been identified in the amniotic fluid of a patient undergoing aminocentesis, and a strain of *Acholeplasma laidlawii* has been identified in the tissues of a stillborn infant. We have also characterized ten other acholeplasmas recovered from a variety of insect and plant origins. Most of these mollicutes have been found to be serologically distinct from all other acholeplasmas, but have the general features (no serum requirement for growth, genome size of $1 \times 10^9$ daltons, G+C value of 30-35 mole%, etc.) of acholeplasmas. Further efforts to understand the ecology of these mollicutes and their occurrence in human tissues appears justified. Additional work on identification of the attachment protein in *Mycoplasma genitalium* suggests that a 143 K protein is involved in adherence of the organism to human tissues, and this protein can be separated from the 165 K protein (P1) identified as the main attachment moiety in *Mycoplasma pneumoniae*. Antibody to the 143 K protein has been identified in the serum of chimpanzees following an experimental genital tract challenge with *Mycoplasma genitalium*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00190-07

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)



| | | | |
|---|---|---|---|
| PI: | Malcolm A. Martin | Chief | LMM, NIAID |
| Others: | Akio Adachi | Visiting Fellow | LMM, NIAID |
| | Roy Repaske | Research Chemist | LMM, NIAID |
| | Arnold Rabson | Medical Staff Fellow | LMM, NIAID |

COOPERATING UNITS (if any)

Paul Steele — Department of Pathology, Washington University School of Medicine, St. Louis, Missouri

Stephen J. O'Brien — Staff Scientist, LVC, NCI

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205


| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 0.6 | 1.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Following infection with retroviruses, a DNA copy of the viral RNA genome is synthesized in a reaction catalyzed by reverse transcriptase. One or two copies of viral DNA is subsequently integrated into the chromosome of the infected cell. If embryos or cells of the reproductive tract are the target of retrovirus infection, a copy of the viral DNA can be inserted into the germline and be transmitted vertically within a given species.

All vertebrates examined to date carry endogenous retroviral sequences. The human genome contains 35 to 50 copies per haploid mass of DNA. These copies are equally divided between full-length (8.8 kb) retroviral structures with LTR, gag, pol and env domains and a truncated class (approximately 4.1 kb in size) containing only gag and pol sequences. Detailed analyses of several human retroviral segments indicate that each contain deletions, stop codons, and frame shifts which render them defective as viruses. On the other hand, long open reading frames abound (pol = 1881 bp; env = 1284 bp). The human endogenous retroviral sequences we have examined possesses two unique structural features that distinguish them from other related retroviral segments: a primer binding site for $tRNA^{Glu}$, and a characteristic env gene. Cross-species, high stringency hybridization analyses have shown that the $tRNA^{Glu}$ class of retroviruses with their distinguishing env gene are present in all primates above old world monkeys.

The number of primary germline insertions of retroviral genomes in human chromosomal DNA is far less than would be suggested by the 35 to 50 copies currently detected, suggesting that amplification of large DNA segments involving both viral and cellular sequences has occurred. We have accumulated data which conclusively demonstrates that such amplification has occurred subsequent to the integration of the $tRNA^{Glu}$ family of retroviruses into the primate germline. Experiments utilizing somatic cell hybrids indicate that the amplified retroviral/flanking cell DNA units have been dispersed to multiple chromosomes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00218-04 LMM

PERIOD COVERED
October 1, 1984 to Septemb»r 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of MCF Related mRNA in AKR Mice (revised title)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Roy Repaske | Research Chemist | LMM, NIAID |
| Others: | Raymond O'Neill | Chemist | LMM, NIAID |
| | Frederic Laigret | Visiting Fellow | LMM, NIAID |
| | Christine Kozak | Microbiologist | LMM, NIAID |
| | Hidetoshi Ikeda | Visiting Fellow | LMM, NIAID |
| | Janet Hartley | Research Microbiologist | LVD, NIAID |
| | Malcolm Martin | Chief | LMM, NIAID |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.95 | 1.95 | 1.00 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The degree of homology of the endogenous human C type proviral sequence compared to C type sequences of Baboon endogenous virus and MuLVs suggested the human provirus represented a new subclass of C type viruses. A probe containing unique human env sequences showed many copies of human env reactive sequences in monkey DNAs. A full-length env reactive provirus was cloned from African green monkey. All regions of this genome sequenced were 80-88% homologous to the human sequences. Analysis of genomic DNA from other old world primates showed all contained human env reactive sequences whereas no new world primate DNA reacted with this probe.

Two 100 bp env specific probes subcloned from the N-terminal gp70 region of MCF and xenotropic MuLVs reacted specifically with the respective MuLV. No cross reactivity was detected with ecotropic or amphotropic proviral DNA. Genomic DNA of laboratory mice unexpectedly contained more copies of MCF related sequences than xenotropic MuLV sequences whereas a more diverse pattern was found in wild mouse strains.

The development of MCF virus was followed in AKR mice from 5 weeks to 6 months of age by the appearance of MCF related RNA transcripts in the thymus and other tissues. Two mRNA species were expressed exclusively in the thymus (1.8 and 7.2 kb), a 6.0 kb species was expressed in liver and kidney and a 3.0 kb RNA was expressed in all tissues. At three months of age the thymus contained a full-length (8.2 kb) MCF reactive message.

Two new defective oncogenic viruses were isolated from a splenic tumor in a C58 V-congenic mouse inoculated with C25 LI MCF. One virus studied mapped as an ecotropic MuLV except for a 1000 by ras sequence which replaced late pol and most of gp70. The DNA sequence of the ras showed amino acid 12 was arginine. The p21 coding segment was closely related to bas, H-ras and Rasheed-ras. The putative recombination site for the 5' crossover was identified.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00219-04 LMM |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular and Genetic Analysis of Streptococci

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Donald J. LeBlanc | Head, BVS | LMM, NIAID |
| Others: | Jon M. Ranhand | Senior Scientist | LMM, NIAID |
| | Julia M. Inamine | Guest Researcher | LMM, NIAID |
| | Graham P. Davey | Visiting Associate | LMM, NIAID |
| | Linda N. Lee | Chemist | LMM, NIAID |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two genetic determinants mediated by a lactose metabolic plasmid from a strain of Streptococcus cremoris, encoding phospho-beta-galactosidase (p-gal) activity and a lactose-specific component of the PEP-dependent phosphotransferase system (lac-PTS), were cloned in E. coli. A cloned 3.5 kb PstI/AvaI fragment transformed mutants of Streptococcus sanguis deficient in lac-PTS and p-gal activiites (lac83), or only p-gal (lac8), to a lactose positive phenotype. Mutant lac8, but not lac83, was similarly transformed by a 2.6 kb SstI/AvaI fragment. Southern blot hybridizations using cloned fragments as probes confirmed the presence of plasmid-specific sequences in the S. sanguis transformants, identified a fragment of S. sanguis chromosomal DNA into which the lactose-specific genes integrated, and established the presence of identical genetic determinants for lactose metabolism in plasmids from S. cremoris and Streptococcus lactis. Incompatibility studies were conducted with naturally occurring deletion derivatives, and with in vitro constructed derivatives, of the broad host-range streptococcal plasmid, pAMbeta 1. The results obtained indicated that genes associated with incompatibility may be located a considerable distance from a previously defined replication region of pAMbeta 1, or that pAMbeta 1 may contain a second origin of replication. A series of transformation experiments with S. sanguis strains containing, or being transformed by, derivatives of the pAMalpha 1 delta 1 family of plasmids were conducted in a study of the regulation of plasmid replication. The results confirmed the requirement for a trans-acting component for the initiation of replication of the pAMalpha 1 delta 1-like replicons, and provided preliminary evidence for negative control of replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00222-04 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Theodore Theodore | Research Microbiologist | LMM, NIAID |
| Others: | Arifa S. Khan | Senior Staff Fellow | LMM, NIAID |
| | Malcolm A. Martin | Chief | LMM, NIAID |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.3 | 1.3 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


One model of leukemogenesis involves the generation of dualtropic murine leukemia viruses (MuLVs) by molecular recombination between the spontaneously induced ecotropic MuLV and endogenous proviral DNA segments present in the chromosomal DNA of the mouse. Following the recombination event, a dualtropic MuLV may gain entry into a susceptible cell (such as a lymphocyte in the thymus) and, if integration occurs at an appropriate site (near putative oncogene), disease may occur. To study the relationship between dualtropic MuLVs and leukemogenesis, we molecularly cloned 2 dualtropic MuLVs; MCF-13 (thymotropic strain) and MCF-111A (non-thymotropic strain) from chronically infected mink cells. Transfection of these cloned DNAs into mink cells resulted in the production of infectious progeny. Nucleotide sequence analyses showed distinct difference in the long terminal repeats (LTRs) between leukemogenic (MCF-13) and non-leukemogenic (MCF-111A) strain. MCF-111A also had a 12 base deletion (encoding for 4 amino acids) near the 3' end of the pol gene. This information will be used to mutagenize the MCF viral DNAs and test their biological activities.

The chloramphenicol acetyl transferase (CAT) gene assay was used to a test for the biological activity of AKR-ecotropic, BALB/c-ecotropic, and NFS-xenotropic LTRs. BALB/c-LTR was devoid of any "CAT" activity whereas NFS and AKR LTRs showed 10% and 3% acetylation, respectively. "CAT" gene constructs are being made with MCF and other endogenous LTRs in attempt to relate the LTRs and their expression of biological activity.


PHS 6040 (Rev. 1/84)



GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00281-04 LMM |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Cloning of Recombinant MuLV Proviral Sequences in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Charles E. Buckler | Research Biologist | LMM, NIAID |
| Others: | Robert A. Yetter | Visiting Researcher | LIP, NIAID |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.6 | 0.6 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Studies with DNAs isolated from 19 MCF247 induced tumors have continued with emphasis on a more complete evaluation of one specific tumor. The 19 tumors divide into three classes with respect to the expression of the Ly-2 surface antigen: 9 of the tumors are Ly-2 +; 3 are Ly-2 -; and the remaining 7 tumors contain a 50-50% mixture of Ly-2 + and Ly-2 - (Ly-2 +/-). Cells from one of the Ly-2 +/- tumors (Ty-3) were inoculated into 5 newborn AKR/J mice. Evaluation of the tumors that developed in these mice showed that one tumor was Ly-2 -, and that the other 4 were again Ly-2 +/-. DNAs isolated from these 5 passage tumors were evaluated for the presence of restriction fragments that hybridize to MuLV ecotropic env and xenotropic env DNA probes. No differences in patterns were observed when compared to DNA from the primary tumor, suggesting that in vivo passage of the established tumor cells is not associated with an alteration in the integrated MuLVs present in the tumor cell genome. Thus a source of material for further evaluation of the role of MuLVs in the development of murine tumors has been established. Further cloning of novel MuLV insertion sequences in these tumors is in progress.

To aid in construction of restriction maps of cloned and genomic DNA, a program was written for the IBM-PC which reduces the effort required to determine the sizes of fragments produced after digestion with restriction enzymes. An improved fitting algorithm, utilizing a cubic spline instead of the more commonly used third order polynominal, results in a more accurate estimation of fragment size.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00300-04 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Control of Resistance to Viral Leukemogenesis in Wild Mouse Populations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Christine A. Kozak    Microbiologist    LMM, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.9 | 0.4 | 0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the genetics of viral leukemogenesis in wild mice have revealed that these mice differ substantially from the inbred strains. Analysis of a variety of feral mouse populations have shown that almost all lack ecotropic-MuLV related sequences. MuLVs with ecotropic host range have been isolated from *M. hortulanus*, *M. m. castaneus* and *M. m. molossinus*. Data from Southern blot hybridizations indicate that most of these viruses differ from ecotropic MuLVs of inbred mice in their hybridization properties and internal restriction maps. Cells of most wild mice also differ from inbred mice in their susceptibility to exogenous infection. Most lack the restriction defined by the known alleles at the *Fv-1* locus. Genetic crosses demonstrated that 2 of these mice, *M. spretus* and *M. praetextus*, carry the rare resistance allele at *Fv-2*. These mice also differ from laboratory mice in their susceptibility to xenotropic MuLVs. This trait is controlled by a single chromosome 1 locus, designated *Sxv* , which may represent a wild mouse polymorphism of the MCF-MuLV receptor locus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00301-04 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Genes Involved in Transformation and MuLV Transmission in Inbred Mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Christine A. Kozak | Microbiologist | LMM, NIAID |
| Others: | James Ihle | | LBI, NIAID |
| | Robert Callahan | | LTIB, NIAID |
| | Darrel Nebert | | LDP, NIAID |
| | Jonathan Silver | | LMM, NIAID |
| | Arifa Khan | | LMM, NIAID |

COOPERATING UNITS *(if any)*

| | |
|---|---|
| P. Tsichlis | Fox Chase Cancer Center, Philadelphia, PA. |
| D. Jolicour | Clinical Research Institute of Montreal, Canada |
| M. Proffitt | Cleveland Clinic Foundation, Ohio |

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.05 | 0.55 | 0.50 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic studies on the transmission of murine retroviruses through the mouse germline, susceptibility to exogenous infection, and virus-induced oncogenis have led to the identification and chromosomal mapping of numerous genetic loci involved in these phenomena. The analysis of somatic cell hybrids has been used either alone or in conjunction with classical Mendelian crosses to map the following genes: mouse mammary tumor proviral loci to chromosome 14 in C3H/HeJ mice and to chromosome 6 in BALB/c and an endogenous MuLV provirus to chromosome 11; the cellular homologs of 3 oncogenes to 3 chromosomes; tumor-specific ecotropic MuLV integration sites to chromosomes 7, 15 and 19; tumor-associated mammary virus integration sites to 3 different chromosomes; and the interferon β-1 genes to chromosome 4. Hybrid cells have also been used to analyze the differential expression of endogenous murine leukemia viral loci in chemically induced hybrid cells which lack the receptors required for secondary infection. The role of the ecotropic cell surface receptor has also been examined in target cell recognition by autoreactive thymocytes.

COOPERATING UNITS (Cont'd.)

| | |
|---|---|
| Stephen O'Brien | LVC, NCI |
| O. Prakish | Sloan Kettering Cancer Center, New York, NY. |
| C. Croce | Wistar Institute, Philadelphia, PA. |
| J. Dudley | University of Texas, Austin, TX. |
| E. Howard | Medical College of Georgia, Atlanta, GA. |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00304-04 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Pathogenesis of Disease Induced by Friend MuLV

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jonathan Silver | Medical Officer | LMM, NIAID |
| Others: | Christine Kozak | Microbiologist | LMM, NIAID |
| | Charles Buckler | Research Biologist | LMM, NIAID |

COOPERATING UNITS (*if any*)
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.80 | 1.30 | 0.50 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Friend helper virus (F-Eco) is a murine retrovirus which can induce a variety of hematopoietic neoplasms. This virus is being used as a model to study mechanisms of leukemogenesis.

1. Genetic Studies: Crosses between AKR and other strains indicated that AKR mice carry at least two genes other than inherited ecotropic retroviruses which predispose them to lymphoma after inoculation with F-Eco. Further genetic studies are being done to localize these genes and determine their relationship to other oncogenes.

2. Molecular studies: Using a provirus-cellular DNA junction fragment cloned from one F-Eco induced myeloid leukemia, we found that several other F-Eco induced lymphomas and myeloid leukemias have an F-Eco provirus integrated in the same region of cellular DNA, designated Fis-1. This common integration region maps very close to Int-2, an oncogene involved in murine mammary carcinomas. On a molecular level, Fis-1, and Int-2 are clearly distinct. Studies are underway to determine if Fis-1 represents a new oncogene involved in lymphoid and myeloid malignancies, or whether F-Eco integration at this site activates Int-2. We are also beginning to investigate the human homologue of Fis-1 which might be involved in human B cell lymphomas, some of which have a translocation involving the region of chromosome 11 which contains the human homologue of Int-2.

3. Significance. F-Eco provides a model system to study molecular events in leukemogenesis. Because of the high degree of conservation of oncogenes, these studies are likely to shed light on genes involved in human cancers.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00353-03 LMM |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biology and Biochemical Structure of Endogenous Proviruses of Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Arifa S. Khan | Senior Staff Fellow | LMM, NIAID |
| Others: | Theodore Theodore | Research Microbiologist | LMM, NIAID |
| | Frederic Laigret | Visiting Fellow | LMM, NIAID |
| | Janet Hartley | Chief, Viral Oncology Section | LVD, NIAID |
| | Joan Austin | Microbiologist | LVD, NIAID |
| | Roy Repaske | Research Chemist | LMM, NIAID |
| | Christine Kozak | Microbiologist | LMM, NIAID |

COOPERATING UNITS (if any)
Charles Rodi — Monsanto Scientist — Chesterfield, MD

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.05 | 1.70 | 1.35 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We had previously isolated several endogenous MuLV proviruses from BALB/c and AKR/J mouse DNAS. Molecular and biochemical characterization of the cloned endogenous MuLV DNAs indicated that about 50% were related to known MuLV proviruses (Class I). Restriction enzyme and nucleotide sequence analysis indicated that the Class I endogenous MuLV DNAs could be distinguished from known infectious MuLV proviruses due to the presence of a transposon-like 190 bp cellular insert in their LTRs and unique restriction sites. Furthermore, the env sequences associated with the majority of such endogenous MuLV DNAs were similar to those present in recombinant MCF MuLVs. In fact, one cloned endogenous AKR MuLV DNA, designated as A-12, was almost identical in sequence of its 5' env region with leukemogenic MCF-MuLV env genes. We have determined the genomic location of the A-12 provirus to be on chromosome 13 or 18. Unlike other endogenous MuLV DNAs, the A-12 endogenous provirus was present in several inbred as well as wild mouse DNAs. Nucleotide sequence comparison of the LTR, 3' pol and env regions of leukemogenic (MCF-13) and non-leukemogenic (MCF-111A) MuLVs indicated a high degree of base homology in the env region. The LTR of MCF-13 was highly related to the LTR present in xenotropic MuLVs whereas, the LTR associated with MCF-111A was identical except for 1 bp with ecotropic MuLV proviral LTRs. The 3' pol region of MCF-13 contained a 12 bp sequence characteristic of leukemogenic MCF MuLVs. This was absent in MCF-111A MuLV DNA. The leukemogenic potential of in vitro constructed recombinant MuLVs was tested. These viruses contained 5' MCF-13 LTR, gag, pol and env sequences and 3' endogenous MuLV env and LTR sequences. Newborn AKR mice inoculated with such recombinant viruses developed thymic lymphomas in about 3 months. Tissue-specific expression of endogenous MCF-related mRNAs was detected in various AKR/N mouse tissues using a $^{32}P$-labeled MCF env-specific synthetic DNA probe. A 7.2 kb mRNA species was identified in the thymus which could be involved in the generation of recombinant thymotropic MCF viruses.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00388-02 LMM |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Human Retroviral Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: Arnold Rabson | Medical Staff Fellow | LMM, NIAID |
| Others: | | |
| Daryl Daugherty | Medical Staff Fellow | LMM, NIAID |
| Malcolm Martin | Chief | LMM, NIAID |
| Thomas Folks | Expert | LIR, NIAID |
| Sundararajan Venkatesan | Expert | LMM, NIAID |

COOPERATING UNITS *(if any)*

| | |
|---|---|
| Esther Chang | USUHS |
| Len Neckers | LP, NCI |

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.65 | 1.65 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


The primary goal of this project is the analysis of the strategies of gene expression utilized by human retroviral genomes and of the functions of the different regions of these genomes. Our studies have focused on two classes of human retroviral genomes; the exogenous human retrovirus associated with the Acquired Immunodeficiency Syndrome (AIDS RV) and endogenous human type-C retroviral sequences related to murine leukemia viruses.

The pattern of gene expression of the AIDS RV during acute infection has been examined by Northern blot hybridization. Viral specific RNAs were detected early in the course of infection, three days prior to the appearances of reverse transcriptase activity in culture supernatant. We have identified five major species of viral RNA of 9.1, 5.5, 5.0, 4.3 and 1.8-2.0 kb. The 9.1 kb RNA hybridized to probes from all regions of the virus and thus represents the full-length viral transcript as well as putative gag-pol mRNAs. The 5.5 and 5.0 kb RNAs hybridized to LTR and "A" region probes as well as probes 3' to "A". The 4.3 kb RNA contained env sequences and the 1.8-2.0 kb family of mRNAs hybridized to the "B" region. Interestingly, the 5.5 kb RNA was not detected in cells producing defective virions.

The expression of human endogenous retroviral sequences has been observed in a variety of human cell types. Human pol sequences are expressed in hematopoietic cells and appear to be inducible in an erythroleukemia line following TPA treatment. Human melanoma cells contain abundant amounts of 3.6 and 2.2 kb "LTR-only" RNAs. Size-fractionation of the melanoma RNA has been carried out in preparation for cDNA cloning. The function of a cloned human $U_3$ LTR (derived from a cDNA clone) is being assessed by ligation of the human LTR to human ras genes. These plasmids will be tested for their transforming potential in NIH 3T3 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00395-02 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Molecular Analysis of Anaerobic Bacteria Indigenous to Humans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: C. Jeffrey Smith Staff Fellow LMM, NIAID

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence

INSTITUTE AND LOCATION
NIAID, NIH, Frederick, Maryland 21701

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular and genetic studies of antibiotic resistance plasmids in the anaerobic bacterium Bacteroides fragilis were performed in order to analyze genetic exchange mechanisms and the dissemination of antibiotic resistance in this important pathogen.

Transposon-like structures encoding clindamycin (Cc) resistance in the three previously described Bacteroides R-plasmids pBF4, pBFTM10, and pBI136 were cloned in E. coli and then characterized by heteroduplex analyses, restriction endonuclease site mapping, and DNA homology studies. Results indicated that all three structures were bounded by a homologous 1.2 kb directly repeated sequence (DRS). The transposon-like elements of pBF4 and pBFTM10 were 5.2 and 5.4 kb in size and shared more than 90% DNA sequence homology. The pBI136 element was 8.4 kb and had diverged significantly; except for the DRS, only 0.85 kb identified as the Cc resistance gene, was homologous to the other two transposon-like elements. This 0.85 kb region was located adjacent to one copy of the DRS.

Thus far antibiotic resistance determinants from Bacteroides have not been found to express their phenotype in any other bacterial genus. To facilitate study of antibiotic resistance transfer, a genetic transformation procedure and gene cloning system were developed for B. fragilis. Through the use of these techniques, the pBI136 Cc resistance gene was cloned and expressed in B. fragilis. Analyses of the cloned DNA indicated that the genes does not possess its own promoter sequence but rather it is regulated by sequences found within the DRS. These results provide significant insight into the means by which Bacteriodes spp. acquire, regulate, and express new genetic information.

PHS 6040 (Rev. 1/84)



GPO 914-918

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00415-02

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
The Molecular Biology of Retroviruses Associated with AIDS

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Malcolm A. Martin | Chief | LMM, NIAID |
| Others: | Theodore Theodore | Research Microbiologist | LMM, NIAID |
| | Akio Adachi | Visiting Fellow | LMM, NIAID |
| | Howard Gendelman | Expert | LMM, NIAID |
| | Steven Benn | Medical Staff Fellow | LMM, NIAID |
| | Daryl Daugherty | Medical Staff Fellow | LMM, NIAID |
| | Thomas Folks | Expert | LIR, NIAID |

COOPERATING UNITS (*if any*)
Hardy Chan, Earl Shelton, Felix Salazar: Syntex Corporation
R1-215 Hillview Avenue
Palo Alto, CA. 94304

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 10.09 | 4.05 | 6.85 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Using techniques of molecular cloning, nucleic acid hybridization and biochemical virology, we have examined the structure of the acquired immunodeficiency syndrome (AIDS) retrovirus and determined the number and types of viral gene products present in infected cells. In collaboration with Dr. Tom Folks (LIR), a continuous $T4^+$ lymphocyte line (A3.01) has been adapted for the examination of biologic and biochemical events associated with cytolytic AIDS virus infection. A3.01 cells have been used for the propagation of the LAV prototype virus and several new North American and African virus isolates. The cell line has also been employed as a source of proviral DNA from which an ensemble of subgenomic DNA probes has been generated.

Labeled nucleic acid probes have been used to monitor productive infection *in vitro* and for the examination of clinical materials. Attempts at using dot, slot, and Northern blot analyses for the direct detection of viral specific nucleic acids in AIDS patients were unsuccessful in view of the paucity of infected cells. *In situ* hybridization approaches, however, have proven useful in demonstrating viral infected lymphocytes and monocytes *in vivo*. Detailed restriction mapping of North American and African AIDS retroviral isolates by Southern analyses of infected cellular DNA has revealed profound genomic heterogeneity. The variation in virus structure is concentrated to the envelope coding region although viral gene sequences located in the 3' half of the viral genome appear to contain more polymorphisms than the *gag* and *pol* segments.

OTHER INVESTIGATORS (Cont'd.)

| | | |
|---|---|---|
| Arnold Rabson | Medical Staff Fellow | LMM, NIAID |
| Sundararajan Venkatessan | Scientist | LMM, NIAID |
| Tom Folks | Expert | LIR, NIAID |
| Clifford Lane | Deputy Director | LIR, NIAID |
| Anthony Fauci | Director | LIR, NIAID |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00433-01 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Use of Retroviruses as Tagged Insertional Mutagens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jonathan Silver | Medical Officer | LMM, NIAID |
| Others: | David Hoggan | Senior Scientist | LMM, NIAID |

COOPERATING UNITS *(if any)*
Cindy Edwards Scientist LDP, NCI

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Viral Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.9 | 0.4 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Proviral insertion into chromosomal DNA is associated with mutation at the site of integration. In systems in which particular mutations can be selected, retroviruses can be used to clone specific cellular genes through the cloning of provirus-cellular DNA junction fragments. With Dr. Cindy Edwards in the Laboratory of Developmental Pharmacology, we are attempting to identify cells in which a Friend murine leukemia provirus has inserted into the gene for the aryl hydroxylase receptor. We plan to use these cells to clone the gene for this receptor. With Dr. David Hoggan (LMM) we are screening cells from humans heterozygous for various autosomal recessive cancer genes to see if amphotropic or xenotropic viruses can be used to identify these genes molecularly.

Significance: These experiments provide a novel strategy for identifying and cloning genes involved in the metabolism of aryl hybrocarbons and in a variety of human malignancies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00434-01 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Dissemination of Streptococcal Antibiotic Resistance Determinants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Donald J. LeBlanc | Head, BVS | LMM, NIAID |
| Others: | Julia M. Inamine | Guest Researcher | LMM, NIAID |
| | Linda N. Lee | Chemist | LMM, NIAID |

COOPERATING UNITS (if any)
Departments of Pathobiology, Epidemiology and Medicine,
University of Washington, Seattle, Washington 98195

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Bacterial Virulence Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 0.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cloned determinants for resistance to streptomycin (Sm), kanamycin (Km) and erythromycin (Em), from the streptococcal plasmid pJH1, were used as hybridization probes to establish the presence of these same determinants in 70% of 91 multiple resistant group D streptococci obtained from human and animal sources. The animal isolates had been obtained from 13 states and the human clinical isolates were from the United States, Thailand and Chile. Five plasmids, three from human and two from animal _Streptococcus faecalis_ isolates, although quite different, contained an identical 9 kb segment that included these Sm, Km and Em resistance determinants. One human clinical isolate of _S. faecalis_ was resistant to high levels (>2000 μg/ml) of spectinomycin (Sp). The resistance was mediated by a novel plasmid-encoded adenylylating enzyme which modified Sp, but not Sm. A 1.1 kb _ClaI/NdeI_ DNA fragment, carrying the gene for Sp modification, was cloned in and expressed by transformants of _Streptococcus sanguis_ and _E. coli_. Cloned fragments were used as hybridization probes to identify, in four Sp resistant group D streptococci of animal origin, and 8 kb segment of DNA identical to a region of the Sp resistance plasmid from the human isolate. Preliminary results indicated that the Sp resistance determinant was present in animal strains prior to its emergence in the human _S. faecalis_ strain. Recent clinical isolates of _Mycoplasma hominis_, resistant to high levels (30 to 100 μg/ml) of tetracycline (Tc), were shown to contain DNA sequences homologous to the streptococcal Tc resistance determinant, _tetM_. This was the first evidence for the presence of _tetM_ in an unrelated genus and suggested the spread of _tetM_ from _Streptoccocus_ to _Mycoplasma_.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00437-01 LMM

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biology and Genetics of the AIDS Retrovirus

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | M. David Hoggan | Senior Investigator | LMM, NIAID |
| Others: | Thomas A. Folks | Expert | LIR, NIAID |
| | John E. Coligan | Research Chemist | LIG, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 0.6 0.5 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is currently being initiated to advance our understanding of the basic biology of the AIDS virus and its interaction with susceptible host cells. The previously reported LAV/Alex cell acute lytic cell system has provided valuable information on many of the parameters of virus/cell interaction such as the kinetics of virus production concomitant with cell killing. We have further studied the morphological evolution of the AIDS retrovirus in these cells using the electron microscope. We also used the electron microscope to show that a newly developed and cloned cell line which had been previously infected with LAV and shown to be Lu-III negative, yet produced LAV RNA and expressed LAV proteins did, in fact, produce large quantities of retrovirus particles. Although these particles would not replicate in susceptible cells and no reverse transcriptase can be detected; these "carrier" cells caused morphological changes in normal susceptible cells when cocultivated with them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00438-01 LMM

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Molecular Biology of Cellular Oncogenes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Eugene M. Santos | Visiting Associate | LMM, NIAID |
| Others: | Angel R. Nebreda | Guest Researcher | LMM, NIAID |
| | Theodore Bryan | Microbiologist | LMM, NIAID |

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Molecular Microbiology

SECTION
Biochemical Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.65 | 0.2 | 0.45 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

ras Gene Amplification and Malignant Transformation. Activating point mutations in ras genes have been identified in about 15% of the most common forms of human cancers. Amplification of otherwise unaltered proto-oncogenes may represent an alternative mechanism of malignant activation. We have carried out experiments aimed at determining whether amplification of ras proto-oncogenes can also lead to malignant transformation. We have demonstrated that the combined effect of multiple copies of the human H-ras-1 proto-oncogene induces malignant transformation of NIH/3T3 cells. We have also reported that amplification of ras genes can be observed in human tumors although at a relatively low frequency. These results show that ras gene amplification occurs in unmanipulated tumor biopsies and, therefore, is not a consequence of *in vitro* establishment of cell lines. Moreover, they indicate that gene amplification is an alternative pathway by which ras can also contribute to neoplastic development.

Monoclonal Antibodies Against ras p21 Proteins. Computer analysis of the predicted amino acid sequences of normal and transforming ras p21 proteins indicates the existence of significant structural differences. These observations raised the possibility that monoclonal antibodies may be elicited against the structural domains specific for transforming p21 proteins. These antibodies could then be used to identify individual cells carrying activated ras oncogenes.

The availability of large amounts of highly purified normal and transforming ras p21 proteins synthesized in *E. coli* has made possible the development of immunization and screening protocols. A panel of positive hybridomas are being currently screened by solid phase radioimmunoassay against purified normal and transforming ras p21 proteins for their ability to exhibit differential affinity for either of the two structural forms of p21.

LABORATORY OF PARASITIC DISEASES

1985 ANNUAL REPORT

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Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

Summary - October 1, 1984 - September 30, 1985

## ADMINISTRATIVE AND ORGANIZATIONAL EVENTS

Thus far LPD has survived the departure of several key administrative personnel of the Institute: Dr. Krause, the Director; Dr. Sell, Scientific Director; Mr. Leasure, Executive Officer, and Mr. Criswell, one of the Intramural administrative officers. LPD is grateful for the solid support which it received from this quartet. LPD hopes, in turn, that it can continue to function in a manner that will contribute to the stability of the intramural research program of the Institute and thereby help the new Director of the Institute, Dr. Anthony Fauci, and the future Scientific Director settle in to their new roles. Dr. Gordon Wallace is currently serving as Acting Scientific Director. Meanwhile, the planning for the future "round robin" move of LPD to a renovated Building 4 continues.

It is always gratifying to see the temporary members of our research staff move on to potentially important institutional positions in which they will have the opportunity to continue their work. Among those from LPD were Dr. John Dame who took a permanent job with the Animal Parasitology Division of the Dep't. of Agriculture at Beltsville and Dr. Kirchhoff who joined the Faculty (Infectious Diseases Div.) at the Univ. of Iowa School of Medicine. Dr. Joanna Hansen left to take a position at the Univ. of Utah Medical School, and Dr. Aust-Kettis returned to an academic post in Sweden. Dr. Celia Maxwill will soon leave, but her future job has not been settled at the time of this report. Dr. Lourdes Muñoz returns to a research position in Mexico and Dr. Steve Aley took a position with the Biomedical Research Institute nearby and continues as a Guest Researcher with LPD. Dr. Oliveira finished his Ph.D. program at Johns Hopkins and returned to Brazil.

Even though the Intramural Labs have labored mightily to achieve personnel reductions (the well-known FTE problem!), LPD seems always to have new research fellows under some program or another. Newcomers to the Malaria Section include Dr. R. Sakai as an Expert to work on mosquito genetics; Allen Saul, a biochemist from Australia as Visiting Associate; and Guest Researchers David Walliker, a British Medical Research Council Staff member to work on genetics of malaria; Michael Good, a physician-immunologist from Australia; Pat Romans, a molecular biologist working on insects; Altaf Lal, a biochemist. Several newcomers to work with Dr. Dwyer are Paul Bates, a Visiting Fellow from the U.K. and Katie Pastakia, a Staff Fellow originally from India. Paul Brindley is a new Visiting Fellow from Australia to be with Dr. Sher's group, and Dr. Renu Lal is a Visiting Fellow from India with Dr. Ottesen. The new Medical Staff Fellows include Richard Finley, working with Dr. Dvorak, Richard Davies and Douglas Ward, both of LCI who will be with Dr. Ottesen's group, and the latest arrival, Rodney Adam, who will work with Dr. Nash.

Foreign travel to carry out field research included Drs. Phil Scott in Brazil and David Sacks in India on leishmaniasis, and Drs. Russell Howard and James Sherwood in the Gambia on malaria. Longer term work assignments

were carried out by Dr. Richard Carter in Australia and New Guinea, and Dr. Eric Ottesen who spent a year in India. Dr. Neva and Albert Gam made a short trip to the Dominican Republic for work on leishmaniasis.

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## HONORS AND AWARDS

Dr. Louis Miller shared in the Paul Ehrlich and Ludwig Darmstaedter Prize for 1985 in Germany, was elected an Honorary Fellow of the Royal Society of Tropical Medicine and Hygiene, and was the invited Dyer Lecturer at the NIH in May 1985.

Dr. Sher serves on one of the TDR/WHO Steering Committees for Schistosomiasis and is co-director of the well-known Biology of Parasitism course given annually at Wood's Hole. He also serves on several Editorial Boards.

Dr. Dwyer serves on the TDR/WHO Steering Committee for leishmaniasis, several Editorial Boards and research granting review Boards for NSF, USAID and WRAIR.

Dr. Dvorak was an invited participant to several WHO sponsored workshops on Chagas' disease research.

Dr. Weinbach continues to serve the Institute as well as the entire NIH scientific community on the overall NIH Library Committee.

Dr. Richard Carter was the recipient of the prestigious Chalmers Medal.

Dr. Neva gave the annual Norman Stoll Lecture to the New Jersey Society of Parasitologists and will serve on the TDR/WHO review of the Chagas's disease program.

Dr. Ottesen chairs the TDR/WHO Steering Committee for Filariasis and serves on the WHO Expert Committee for Onchocerciasis, the Steering Committee for the Onchocerciasis Chemotherapy Fund and the advisory committee of the Edna McConnell Clark Foundation.

Laboratory of Parasitic Diseases

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## RESEARCH PROGRESS

IMMUNE RESPONSE

B- and T-cell immunoregulation: Since elevated IgE levels and certain allergic

IN FILARIAL AND OTHER NEMATODE INFECTIONS

manifestations often are seen in human filarial infections, factors affecting immunoregulation are of particular. interest. Improvements were made on a system for studying production of parasite-specific IgE in-vitro that was described last year. The lymphokine that induces parasite-specific IgE production by normal human B-cells was partly characterized as a heat-labile, mannose-rich 10-30 Kd glycoprotein (Nutman, Volkman, Ottesen). It also appears that an EBV transformed cell line can produce parasite-specific or non-specific IgE antibodies (Nutman and Volkman). The antigen-specific T-cell suppression seen in filariasis patients with microfilaremia was found to extend to production of the lymphokines Interleukin-2 (IL-2) and gamma-interferon (γ-INF). Neither lymphokine was produced when lymphocyte cultures from such patients were exposed to filarial antigen, but both IL-2 and γ-INF were produced on exposure of the cultures to mitogens or non-filarial antigens (Nutman and Ottesen). This finding may allow the phenomenon of parasite related immunosuppression to be examined more closely at the T-cell activation stage. The sub-class of IgG found in serum of patients with filariasis that "blocks"IgE-mediated hypersensitivity reactions is being defined more precisely. A battery of 30 anti-subclass monoclonal antibodies that can be used as reagents were analyzed vs. nearly 40 human IgG myelomas. The fact that individual sera from those filariasis patients whose immunoblots with IgE show strong recognition of the same filarial antigens as their IgG4 antibodies further implicates the latter subclass as the blocking antibody (Hussain and Ottesen). These findings also suggest that a fundamental linkage between these two isotypes of Ig exists at the T-cell level.

Disease characterized by immune response. The technique of spreading out parasite antigens by electrophoresis through polyacrylamide gels, "blotting" them onto cellulose acetate paper, exposing these blots to patient's serum and finally identifying those antigen bands reacting with the antibody via radio-labelled reagents is referred to as immunoblotting. The patterns of immunoblotting to filarial antigens shown by filarial patients with different clinical manifestations of filariasis is helpful in understanding the immunological basis of the disease (Hussain and Ottesen). This same procedure may prove helpful for purposes of immunodiagnosis if species-, genera- or stage-specific parasite antigens can be developed. A more sensitive assay for detection of circulating filarial antigen is also receiving attention (Lal, Hussain, Nutman and Ottesen). The broncho-alveolar lavage studies of patients with tropical pulmonary eosinophilia (TPE) have shown that a chronic low-grade alveolitis persists in most patients for months after a standard course of DEC therapy. Therefore, trials of some further treatment (longer DEC or burst steroids) are planned (Ottesen, Crystal, Nutman). Tests for additional potential immunologic mediators of lung damage in lavage fluids from TPE patients are planned in an effort to identify how the lung damage occurs (Nutman, Rom, Pinkston, Ottesen and Crystal). Exam of eye tissues recovered during cataract surgery from 20 Ghanian patients shows abundant suppressor T-lymphocytes in onchocerciasis patients, in contrast to a preponderance of helper T-cells in most cases of non-parasitic uveitis (Nussenblatt, Francis and Ottesen).

Strongyloidiasis and hookworm: Experience with both the somatic and metabolic larval antigens of *S. stercoralis* confirms their usefulness as immediate skin test antigens in diagnosis of human infection with this

parasite. Cross reactivity with the related rat parasite, S. ratti, extends only to the somatic and not the metabolic antigens, indicating greater specificity of the latter. Thus far the only false positive reactions have been encountered in patients with occult filariasis, but a wider experience with cases of nematode infections especially is needed (Neva and Maxwell). Results of immediate skin test reactions, as well as the ELISA test for serum antibody, in 21 American ex-POWs held in S.E. Asian prison camps during WW II provided very good correlation of these two tests in diagnosis of strongyloidiasis (Neva and Pelletier of VA). Presence and relative abundance of intestinal mast cells was found to correlate with control of experimental strongyloides infection in the Patas monkey, being increased in chronic infection and virtually absent after administration of steroids and hyperinfection (Barrett, Neva and London). Experimental infections with hookworm (N. americanus) in normal volunteers with 50 larvae resulted in a vigorous eosinophil response, but minimal specific humoral and cell-mediated responses (Ottesen, Maxwell, Hussain and Nutman).

IMMUNOLOGY OF SCHISTOSOMIASIS

Genetics of immunity in mice: Previous work on defective immunity in P strain mice was tentatively linked to defective killing of schistosomulae by activated macrophages. A single gene defect was also demonstrated to account for defective vaccine-induced immunity to A/J mice, and the defect was tentatively mapped to chromosome 6. Genetic complementation experiments showed that the gene locus controlling the defect in A/J mice is distinct from that controlling the defect in P strain mice (Oliveira, James and Sher). A different aspect of genetic control of experimental schistosomiasis in mice has been examined--namely, possible differences in the immunizing capacity (by previous infection) of different strains of the parasite, instead of the host. Two strains of parasite recovered from the same patient, and used to infect mice, were found to produce markedly different degrees of resistance to subsequent re-infection. When both parasite strains were irradiated, comparable degrees of resistance to re-infection was induced by the two strains. Obviously, some other mechanism of immunity that requires living parasites must be operating with one of the strains of parasites (Cheever, Malley of DCRT).

Schistosome age may be critical for vulnerability to macrophage attack as well as presence of important antigen: Previous work has clearly shown that very young (few hours) schistosomulae are highly susceptible to killing by activated macrophages, but that they become refractory by several days of age, and it has been assumed that they remain refractory. However, a "window" of susceptibility to killing by activated macrophages, at least under in-vitro conditions, was found in 2 1/2 week old worms recovered from infected mice. This property of worms may also correlate with permeability of the worm's tegument induced by the chemical, tetraphenylphosphonium, used as a probe of membrane function (Pearce, Zilberstein, James and Sher). A 97,000 MW protein antigen, extracted from schistosomes, but not present on the surface membrane, appears to be a protective antigen. Animals immunized with worm extracts plus BCG and protected against challenge, recognize this protein when analyzed by Western blots. Also, the fraction of worm extract containing this antigen was able to induce protective immunity. Attempts to further purify the antigen and determine its animo acid sequence are in progress so that an oligonucleotide probe might be prepared for cloning the gene that encodes the protein (Pearce, James and Sher). The problem of

identifying recombinant DNA clones that express relevant schistosome antigens has turned out to be more difficult than expected. Screening of a cDNA library with antisera from immune mice failed to reveal reactive clones, but sera from immunized rabbits were positive. The hypothesis now is that antigenic gene products that should have been recognized by mouse antibodies have been destroyed because of their expression within the β-galactosidase molecule. So, the search goes on, using rabbit antisera as screening reagents, looking especially for clones expressing the 97K schistosome antigen (Lanar, Pearce, Sher and McCutchan).

New look at old issues in schistosomiasis: From various types of evidence it has long been assumed that the reason schistosome worms recovered from experimental hosts lack surface antigens detectable with antisera is because the parasite surface tegument is covered or masked by host molecules in-vivo. This issue was re-assessed by injecting in-vitro grown worms (surface antigen present) intravenously into mice, then recovering and assaying them for expression of surface parasite and/or host antigen. Evidence from this type of experiment, including use of $^{125}I$ labelled worms, suggest that surface antigens are shed rather than masked by host molecules (Pearce and Sher). The anodic schistosome antigen from the gut of the worm has usually been impossible or very difficult to detect in the circulation of infected patients, presumably because it is complexed with antibody, or cleared in some manner from the circulation. New attempts to detect this antigen in the circulation are being made with a modified ELISA test (Lunde and Nash). Using several combinations of mouse and parasite strains, inconsistency between size of granulomas around schistosome eggs and degrees of fibrosis in the liver is another type of evidence that the etiology of hepatic fibrosis in this disease is not entirely clear (Cheever).

IMMUNOLOGY OF LEISHMANIAL INFECTION

More on the infective stage of leishmania: Evidence has been extended that organisms in the stationery phase of growth, in contrast to log phase, are the infective form, whether in culture or in the vector sandfly. Log phase organisms bind to the lectin, peanut agglutinin, whereas the stationery or infective forms do not, thereby providing one way to purify the infective stages. Polyvalent antisera from rabbits immunized with such forms and used for Western blots and immuno-precipitation identified a 116,000 MW antigen found only on the infective form, which now deserves to be called a metacyclic promastigote. One monoclonal antibody has been obtained which is reactive primarily with the metacyclic parasite (Sacks and Sher).

Experimental immunization of mice: Additional aspects of the immunity reported by others to be induced by intravenous inoculation of irradiated promastigotes were investigated. A variety of inbred mouse strains can be protected, high antibody levels develop but delayed hypersensitivity does not develop. Attempts to define protective antigenic fractions that are soluble have been inconsistent. The effect of μ-suppression (prevents development of B-cells) on development of immunity to *L. major* infection was studied in the resistant C3H mouse instead of the susceptible BALB/c, as was done before. Again, B cells and/or antibodies were found to be necessary for normal T-cell immunity to develop (Scott, Sher and Sacks).

Clinical studies: Over the past few years a considerable number of patients with various forms of cutaneous leishmaniasis diagnosed and treated at the Clinical Center have also had assessment of their cell-mediated immune (CMI) responses to mitogens, specific and non-specific antigens. In an effort to evaluate these CMI responses in greater depth and correlate them with other CMI responses, we have also been assaying interleukin-2 (IL-2) and gamma interferon (γ-INF) production by lymphocyte culture in such patients. Generally, lymphocytes of patients with normal immune function are able to produce IL-2 and γ-INF in response to mitogen and antigen stimulation. Patients with diffuse cutaneous leishmaniasis (DCL), who are specifically anergic to leishmanial antigen, also fail to produce IL-2 and γ-INF, as would be expected (Neva, Scott and Sacks). Another approach to evaluating CMI is to select in a positive manner those lymphocytes to be tested with antigens. This can be done by rosetting out all T-cells, and subsets of T-cells with monoclonal antibody, to then test unfractionated lymphocytes, all T-cells and T-4 (helper) cells. These techniques have also been used on patients with visceral leishmaniasis in India who exhibit antigen specific immunosuppression during acute disease, with later recovery of T-cell responsiveness. Thus far, the kala azar cases show the same type of immunologic defect as the DCL patients (Sacks and Indian collaborators). Because γ-INF reportedly can activate macrophages infected with several other intracellular pathogens (as toxoplasma), a trial of recombinant γ-INF therapy was carried out on two DCL patients. Even though some clinical improvement in lesions was noted the concentration of viable parasites in lesions showed only a minimal reduction (Neva and Lane of LIR).

LEISHMANIAL BIOCHEMISTRY

Immunogenicity of membrane antigens: Immunoblotting of L. donovani membrane antigens that have been transferred to paper from gels show that kala azar patients from diverse geographic areas (Africa, India and Brazil) show qualitatively similar patterns. This indicates that the broadly common antigens are highly immunogenic and are common to amastigotes as well as promastigotes. The same findings pertain to soluble antigens released during log-phase growth of the parasites (Dwyer). Some of the ganglioside glycolipids found on L. donovani membranes are probably antigenic (Dwyer and Wassef). The soluble acid phosphatases released by growing parasites were purified and partially characterized; additionally, a system of isotope pulse-labelling with $^{35}$S-methionine was developed to study synthesis of the enzyme (Dwyer, Gottlieb at Hopkins and Bates). Using photoaffinity labelling methods, the glucose transport protein on the surface membrane of L. donovani promastigotes was identified and is currently being purified (Zilberstein and Dwyer). Evidence was found for a ribose transport system in the parasite, presumably driven by a transmembrane electrochemical gradient (Pastakia and Dwyer). Since promastigotes of leishmania are very sensitive to destruction by the alternate complement pathway, the kinetics of $^{125}$I-labelled C3 binding are under study. A putative acceptor target protein for this C3 binding of about 140 KD has been identified (Puentes and Joiner of LCI and Dwyer). The cloning of genes for various surface membrane products of L. donovani promastigotes is being approached by isolation of mRNA attached to parasite polysomes (Bates and Dwyer).

T. CRUZI AND CHAGAS' DISEASE

Models for population dynamics of clones: Since it is now clear that an isolate of T. cruzi from an infected bug or even from a patient consists of a mixed population of genetically stable clones, it is logical to examine what will happen to relative proportions of individual clones when mixed and passaged in nature. This can best be done by computer simulation, knowing the relative proportions of starting populations and the growth rates of each population (Dvorak). Consideration of such population dynamics of T. cruzi during passage in vector bugs and mammalian hosts in nature has important implications in the epidemiology of Chagas' disease. A tubercidin-resistant T. cruzi clone has been developed following mutigenization with ionizing radiation and selection by growth in medium with high concentrations of tubercidin lethal to the parent stock (Finley and Dvorak). A MEDLARS-based, computer-processed bibliography on Chagas' disease covering the years 1968-1984 and containing over 2400 entries was produced. Each entry was classified into up to 12 subject areas and the complete bibliography will be published by the Pan American Health Organization (Dvorak, Gibson and guest researcher Maekelt).

Various T. cruzi clones in animals: Detailed studies of the variable biologic behavior of 3 or 4 distinct clones of T. cruzi in mice, and the importance of this finding to concepts about pathogenesis of the chronic human disease, have been referred to in previous reports. This work has now been extended by examining the course of infection in two different in-bred strains of mice (C3H and C57Bl/6) infected with 14 clones derived from a population of parasites isolated by xeno-diagnosis of a human cases of chronic megacolon disease in Brazil. The resultant infections showed early parasitemia with all clones, high mortality with others, and virtually any result with at least one of the 14 clones. It was also shown that in-bred Lewis rats exhibit essentially the same disease patterns with clones of T. cruzi as are seen in mice (Postan and Dvorak). By modification of electrocardiographic equipment to slow down the recorded rate of the mouse, it has been possible to obtain EKGs on infected mice for periods up to one year. Conduction abnormalities do occur, and with at least one clone a different and more abnormal EKG pattern develops in the infected mice over time (Postan, Dvorak, and Bailey and Pottala of DCRT). The implications of these findings for a suitable small animal model of chronic 'Chagas' disease are obvious.

Interactions of complement and action of monoclonal antibodies with T. cruzi: The kinetics of binding of different fractions of the C3 component of complement to T. cruzi during activation of the alternate pathway was studied with radiolabelled fractions (Joiner of LCI and Sher). The culture-generated form of T. cruzi metacyclic trypomastigote (CMT) was found to lack an epitope, GP72, recognized by a monoclonal antibody, (Mab), whereas epimastigotes and insect-derived metacyclic types had this epitope. The GP72 epitope is of significance because it is the major target antigen of complement. Nevertheless, the presence of the GP72 could be demonstrated on CMT by Iodogen surface labelling and immunoprecipitation. It is hypothesized that GP72 undergoes some conformational change or is covered by other molecules to render it insusceptible to action of complement (Kirchhoff and Joiner of LCI). Anti-idiotypic antibodies raised against a Mab (WIC 29.26) that reacts with the GP72 epitope also recognized this epitope on epimastigotes. However, immunization of mice with this Mab failed to protect

the animals against subsequent challenge with metacyclic trypanosomes (Sacks, Sher and Kirchhoff).

New serologic test based upon specific antigen epitopes: Based upon the assumption that all strains of *T. cruzi* possess one or both antigenic epitopes (72 and 90K) that have been identified with Mabs, a serologic test for antibodies to them epitopes was developed. The test involves radiolabeling the parasite surface antigens, immuno-precipitation with test serum, electrophoresis and identification of the reactive antigens by auto-radiographic labelling. Although the test is too complex for routine application, it appears to be the most specific and useful in differentiating cross reactions that would occur with other tests. Antibodies to the 72 and 90K antigens were present in parasitologically proven cases from diverse geographic locations, and the antigens are also present on parasite strains from widely separated regions (Kirchhoff and Neva).

Anti-trypanosomal factor from Pseudomonas flourescens: Further purification was achieved of the protein (ATF-II) with greatest activity against *T. cruzi*. Anti-trypanosonal activity was present in fractions containing 10-30μg of ATF-II protein. A commercial antibiotic, Viscosin, derived from *P. viscosa*, probably has a similar chemical structure to that of ATF-II, but does not appear to be as effective in controlling *T. cruzi* infection in mice (Mercado and collaborators).

IMMUNOCHEMISTRY AND MOLECULAR BIOLOGY OF MALARIA

Is thrombospondin responsible for P. falciparum cytoadherence to endothelial cells? Thrombospondin, a protein synthesized by endothelial cells and associated with aggregating platelets on vascular surfaces, was fortuitously found to be able to bind knob positive (K+) but not knob negative (K-) *P. falciparum* infected red cells. This was because the same melanoma cell line used for previous studies of *P. falciparum* binding and inhibition of binding with certain anti-malarial antibodies synthesizes thrombospondin. The binding of K+ infected red cells can be demonstrated on plastic surfaces coated with thrombospondin, and binding to the melanoma cell line can be inhibited with anti-thrombospondin antibody or by soluble thrombospondin. Further information on the role of thrombospondin in the phonomenon of deep vascular schizogony of falciparum malaria *in vivo*, its relationship, if any, to the histidine-rich proteins, etc. will be of great interest (Sherwood of LPD, Roberts, Spitalnick and Ginsburg of NIADDK, and Howard and Miller of LPD).

Histidine-rich and other proteins associated with cytoadherence: The story on proteins associated with knobs and cytoadherence of *P. falciparum* red cells remains complicated even though monoclonal antibodies (Mabs) and other properties clearly differntiate two separate histidine rich (His RP) proteins. One is associated with presence of knobs and is insoluble, the other is made by both K+ and K- parasites, is released from parasitized cells throughout growth into the plasma of the host and binds strongly to bivalent metal cations. Using Mabs, polyvalent serum and an oligonucleotide probe, a lambda GT11 exprssion library of *P. falciparum* is being screened to identify clones that would lead to isolation of the gene for the soluble His RP (Wellems, Panton and Howard). Certain halogenated histidine analogues are able to inhibit morphological development and protein synthesis of *P. falciparum* (Panton and Howard). One large cell surface protein ($M_r$ about 300,000) is present only on K+ falciparum infected red cells. The

other large protein of approximately the same size is associated with the submembrane cytoskeleton (Howard, Aley and collaborators).

Antigenic diversity of P. falciparum: Using a new micro-agglutination assay and IFA, evidence was found for multiple P. falciparum antigens on infected red cells of Gambian children. Antibody specific for the infecting strain of parasite, but not reactive with parasites from other patients, was demonstrated in convalescent sera of patients. However, sera from presumably immune adult Gambians reacted with all or most of the isolates. Preliminary evidence that there may be a conserved epitope(s) on P. falciparum infected erythrocytes was obtained from results of adsorption and elution experiments of adult sera with infected red cells (Sherwood and Howard plus Marsh of Gambia MRC).

Antigenic variation after immunization with merozoite antigen: Last year's report of failure of protection vs. P. knowlesi after immunization with a merozoite protein antigen failed to differentiate between antigenic variation or selection of a variant already present in the challenge inoculum. When animals immunized with the $M_r$ 140,000 merozoite antigen were challenged with cloned parasites, variants appeared that were resistant to antibodies that blocked invasion of the parent strain. Passage of the mutants in splenectomized monkeys resulted in loss of expression of the $M_r$ 140,000 variant, but the parent line showed no change in expression of this protein antigen when passed in splenectomized monkeys. These findings do not augur well for the future of a merozoite malarial vaccine (Klotz and Miller).

Genetic manipulation of malaria parasites and vector mosquitoes: Work has been continued on the use of mung bean nuclease and the conditions under which it cuts genomic DNA of the malaria parasite. In addition to the circumsporozoite protein of P. falciparum reported last year, this reagent appears to also cleave coding areas for Plasmodial actin, histidine rich protein, ribosomal RNAs and tubulin (McCutchan, Hansen and McNicol). In an effort to develop new tools by which malaria might be controlled, methods are being developed for introducing genes into vector mosquitoes. It was found that mosquito eggs could be injected directly within one hour of laying and still embryonate and hatch, so direct injection of DNA into eggs will be one method tried. Several different markers selected for genetic studies include larval resistance to neomycin and resistance to dieldrin or DDT (Miller, Sakai and Romans). Further genetic crossing experiments with the lines of An. gambiae refractory to P. cynomolgi indicate that resistance is controlled by more than one gene (Gwadz and Sakai).

Immunogenicity of malaria sporozoites: We are collaborating with investigators at WRAIR and the commerical firm of Smith, Kline and French to test the suitability of a recombinant DNA circumsporozoite antigen of P. falciparum as a human vaccine. The recombinant CS protein is antigenic for mice even without adjuvant, but is more effective in producing antisporozoite antibodies when combined with alum. Availability and immunogenicity of the CS antigen warrant Phase I trials of safety and potency in humans in the near future (Miller, Gwadz and Collaborators).

Transmission blocking immunity to malaria parasites: Last year's claim that only a single epitope on gametes of P. falciparum were recognized by Mabs was incorrect because antigens were separated under reducing conditions

in SDS-gels. Under non-reducing conditions two smaller epitopes on gametes react with transmission blocking Mabs (Carter, Kumar and Collaborators). An expression library of mung bean nuclease digested genomic DNA of P. falciparum in lambda gt-11 phage has been screened with various antisera. Several promising clones have benn selected for further analysis (Kumar, McNicol and McCutchan). Different isolates of P. falciparum show variable degrees of infectivity to selected species of Anopheline mosquitoes (Quakyi). Genetically distinct clones of P. falciparum are being hybridized in genetic recombination experiments during transmission through mosquitoes and primates (Walliker, Carter and Quakyi). In studies on P. vivax infections in Sri Lanka it has been shown that even a primary infection in humans results in development of considerable transmission blocking immunity. From human infections as well as with Mabs it appears increasingly that transmission blocking with P. vivax varies among different isolates of the parasite (Carter and K. Mendis of Sri Lanka).

IMMUNOLOGY AND BIOCHEMISTRY OF LUMINAL PROTOZOA

Giardia and Cryptosporidia: Analysis of antigenic differnces among strains of Giardia was extended to use of Mabs and immunoblotting, as well as DNA restriction patterns, surface labeling and other techniques reported last year. One Mab was shown to be cytotoxic to the parasite. Although antigenic analysis allows parasite strains to be placed in distinct groups, these groupings do not correspond to sources of origin, i.e.-human or animal (Nash, Keister, Agarwal). With the help of collaborators at the Beltsville Animal Diseases group, cryptosporidial oocysts from infected calves were made available to prepare antigen for use in serological tests. An ELISA test was developed which detects both IgM and IgG class antibodies in infected humans. Accumulating evidence indicates that this intestinal protozoan parasite, hitherto thought restricted to animals, is also a significant cause of diarrheal disease in immunocompetent as well as immunologically compromised hosts (Ungar, Nash and Collaborators at Beltsville and N.Y. City). A new method for isolation of calmodulin, a regulating protein for Ca++ metabolism, was developed. Purified calmodulin from Giardia trophozoites appears to have an amino acid composition different from that of calmodulin from other animal species (L. Muñoz and Weinbach). The mechanism of action by which chlorimpramine inhibits in-vitro growth of Giardia is still not clear, but it does not involve blockade of membrane ATPase or glucose uptake as was shown for L. donovani (Zilberstein and Weinbach).

Studies with E. histolytica: Yeast extract and serum required for axenic growth of E. histolytica have been replaced with a mixture of more well defined ingredients (Diamond). Some sucess in conversion of avirulent E. histolytica to a virulent strain, and suppressing virulence was achieved by manipulation of the bacterial flora (Mirelman, Aust-Kettis and Diamond). Further evidence for differences between E. histolytica and the Laredo-type amebae was obtained by comparing ribosomal cistrons of the two organisms with that from a Plasmodial species and by comparison with tubulin probes from a leishmanial species (Diamond in collaboration with Wirth and French of Harvard).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00094-26 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Lumenal Protozoa: Nutrition, Differentation, Virulence, DNA Hybridization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | L. S. Diamond | Section Head | LPD, NIAID |
| Others: | A. Aust-Kettis | Guest Researcher | LPD, NIAID |
| | David Mirelman | Guest Researcher | LPD, NIAID |
| | D. B. Keister | Biologist | LPD, NIAID |

COOPERATING UNITS *(if any)*

Department of Tropical Public Health, Harvard University (D. Wirth and C. French)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Growth and Differentiation

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 2.3 | 2.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A semi-defined medium has been developed for axenic cultivation of a strain of *Entamoeba histolytica*. The only undefined ingredient is a 0.5% of caesin peptone digest. The serum component of TYI-S-33, a medium currently used worldwide for axenic cultivation of *Entamoeba*, has been replaced with a lipoprotein-cholesterol fraction of bovine serum and a commerically produced chemically defined serum substitute. Yields of amebae are equivalent to those obtained with whole serum. Conversion of an avirulent stain of *E. histolytica* characterized by a non-pathogenic zymodeme and isolated from an asymptomatic carrier case to a virulent strain was accomplished for the first time by suppressing the growth of the intestinal flora associated with the amebae and transferring the amebae to a medium for axenic cultivation. Conversely, the virulence of a highly virulent axenized strain of *E. histolytica* was suppressed when the ameba was associated with the bacterial flora derived from the avirulent strain. Reaxenization was followed by restoration of virulence. New evidence that *E. histolytica* and *E. histolytica*-like Laredo type amebae are not conspecific has been provided by comparing the ribosomal cistrons of the two organisms probed with the ribosomal cistron of *Plasmodium lophurae*.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00097-27 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | T. I. Mercado | Research Physiologist | LPD, NIAID |
| Others: | V. Ferrans | Chief, Ultrastructure Sec. | PB, NHLBI |
| | H.D. Hochstein | Chief, Biological Testing and Reference Standards Branch | DCA, BOB |
| | K.C. Rice | Research Chemist | LC, NIADDK |
| | L. Amende | Senior Staff Fellow | LCDB, NIADDK |

COOPERATING UNITS (if any) Waters Associates, Rockville, Md. (M.P. Strickler); Sumitomo Chemical Co., LTD., Osaka, Japan (S. Ogino); Fermentation Unit (H. Hearn) and Shared Services Dept. (J. Beutler); Frederick Cancer Res. Fac. NCI; Dept. of Medicinal Chemistry, Univ. of Illinois, Urbana (K. Rinehart)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on an anti-trypanosomal factor from the bacterial species, Pseudomonas fluorescens, disclosed that extraction procedures employing ether and ethanol as well as chromatographic techniques with hydrophobic columns were very effective for the purification of the lytic substance. The elucidation of the hydrophobic and hydrophylic components will be the subject of further studies on the chemical structure of this compound. The aim of the project is to synthesize the active factor, examine its chemotherapeutic potential in experimental infections with Trypanosoma cruzi, and determine its mechanism of action.



PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00098-29 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | E. C. Weinbach | Section Head | LPD, NIAID |
| Others: | L. Munoz | Visiting Fellow | LPD, NIAID |
| | C. E. Claggett | Bio. Lab. Tech. (Chemist) | LPD, NIAID |
| | S. C. Wieder | Bio. Lab. Tech. (Chemist) | LPD, NIAID |
| | L. Levenbook | Research Chemist | LPB, NIADDK |

COOPERATING UNITS (if any)
Department of Biochemistry, University of Stockholm (B.D. Nelson, T. Hundal)
Ciba Pharmaceutical Company (J.L. Costa)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Physiology and Biochemistry

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4 | 2 | 2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Continued studies on the isolation and characterization of calmodulin from Giardia lamblia have led to a novel method for the rapid isolation of this protein involving heat denaturation of non-calmodulin proteins and anion exchange chromatography. Characterization of the purified calmodulin show it to be as effective as mammalian calmodulin in activating cAMP phosphodiesterase, and identical in its isoelectric properties. In contrast, the amino acid composition of the parasite calmodulin differed markedly from that of known calmodulins. Studies of the ATPases of G. lamblia showed that the Mg2+-activated enzyme was insensitive to calmodulin antagonists, whereas the Ca2+-dependent enzyme was strongly inhibited. Modulation of the Ca2+-ATPase may be an important physiological function of calmodulin in this parasite. Continued studies on the effects of tricyclic antidepressant drugs on parasitic protozoa disclosed that chlorimipramine suppressed growth of G. lamblia. The mechanism of inhibition has not been elucidated, but does not involve suppression of glucose uptake, as was demonstrated for Leishmania donovani by Zilberstein and Dwyer. Studies on mammalian bioenergetics focused on human platelets. Tricyclic antidepressant drugs partially suppressed the burst of oxygen uptake induced by thrombin. The drugs had no effect on the mitochondrial portion of the burst, but presumably inhibit the oxygenases that catalyze the biosynthesis of prostaglandins. Use of imipramine analogs demonstrated that compounds most effective in inhibiting platelet function were those most effective in blocking bioenergetic phenomena in rat liver and beef heart mitochondria, suggesting a common mechanism of action.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00099-15 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. A. Dvorak | Res. Microbiologist | LPD, NIAID |
| Other: | M. Postan | Visting Associate | LPD, NIAID |
| | G. L. Widmer | Guest Worker | London Sch. Trop. Med. Hyg. |
| | R.W. Finley | Med. Staff Fellow | LPD, NIAID |
| | C. C. Gibson | Engineer | BEIB, DRS |
| | W. S. Schuette | Engineer | BEIB, DRS |
| | G. A. Maekelt | Guest Worker | Universidad Central de Venezuela, Caracas |

COOPERATING UNITS (if any)

Harvard School of Public Health, (R. Hoff); LAS, DCRT (J. Bailey, E. Potala)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


This project is concerned with studies of the genetic diversity of Trypanosoma cruzi and the implications of this diversity in the presentation and course of Chagas' disease. Major emphasis during the year has centered on four topics: 1) Utilization of the T. cruzi data base and results of multivariant analysis to model and predict the population dynamics of mixtures of clones; 2) Analysis of the patterns of presentation and disease in inbred mice infected with T. cruzi clones; 3) Development of mutant T. cruzi clones for the production of T. cruzi hybrids; 4) Analysis of the inter-relationship between environmental temperature and the respiratory enzymes of T. cruzi clones.

The flow cytometer development program is nearing completion. The instrument has been equipped with quartz optics to permit analysis in the deep UV and Coulter volume orfice and electronics. The performance of the Coulter volume system exceeds all previous attempts to incorporate this parameter into a flow cytometer. This has proven to be an important development for the analysis of cells with unusual symmetry such as Giardia lamblia.

A MEDLARS-based, computer-processed bibliography of Chagas' disease (1968-1984) has been completed.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00102-11 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | F. A. Neva | Chief | LPD, NIAID |
| Others: | D. Sacks | Senior Staff Fellow | LPD, NIAID |
| | P. A. Scott | Staff Fellow | LPD, NIAID |
| | C. Lane | Senior Investigator | LIR, NIAID |

COOPERATING UNITS (if any) University of Arizona, Tucson, AZ (E. A. Petersen); Harvard Medical School, Boston, MA, (F. vonLichtenberg); Armauer Hanson Research Institute (AHRI), Addis Ababa, Ethiopia, (G. Bjune); Institute of Dermatology, Santa Domingo, Dominican Republic (H. Bogaert).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology and Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 0.7 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


This project continues to focus upon leishmaniasis. The studies involve characterization of parasites recovered from patients, use of experimental models of leishmanial infection in genetic in-bred strains of mice, and examination of the immune response in humans with various clinical forms of leishmanial infections.

In addition to the group of *L. aethiopica* isolates from recently studied cutaneous disease cases from Ethiopia, we have accumulated 6 further isolates from widely scattered regions of the world (Peru, Dominican Republic, Afghanistan and Morocco, which are in process of characterization. Some exceptions to the original general pattern of heat sensitivity of species has turned up; one member of the *L. mexicana* complex and the *L. aethiopica* isolates were found to be sensitive to 35°C. Our sequential studies of the histopathology of leishmanial lesions in the ears of BALB/c mice are continuing, and we have confirmed late metastatic spread of *L. m. amazonensis* in the resistant C3H mouse with a cloned isolate of the parasite.

Work on the cell-mediated immune response in humans with leishmanial infection has comentrated mainly on assay of interleukin 2 (IL-2) and gamma interferon (gamma-INF) produced in lymphocyte cultures. We have also started to generate these lymphokines, along with lymphocyte blastogenesis to mitogens and antigens, using lymphocyte subsets --namely, unfractionated, total T cells and T 4 cells. A trial of gamma-INF therapy in two patients with diffuse cutaneous leishmaniasis is underway, but final results are not yet available.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00103-18 LPD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. N. Lunde | Research Zoologist | LPD, NIAID |
| Others: | A. W. Cheever | Assistant Chief | LPD, NIAID |
| | L. Jacobs | Scientist Emiritus | NIAID |
| | T. E. Nash | Senior Investigator | LPD, NIAID |
| | H. Masur | Deputy Chief | CCM, CC |
| | M. G. Pappas | Dept. Immunology | WRAIR |

COOPERATING UNITS *(if any)*
Critical Care Medicine, Clinical Center, NIH
Department of Immunology, WRAIR

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.1 | 0.4 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The relationship between Toxoplasma gondii and cerebritis in AIDS patients was studied. Toxoplasma was found to be the most frequently recognized cause of cerebral mass lesions in AIDS. Toxoplasma was demonstrated in 7 of 22 (37%) of AIDS patients having an anti-toxoplasma titer equal or greater than 1:256. Specific antigenic determinants on the surface of Toxoplasma cystozoites appear to be lost after a brief period (3 days) of multiplication within skeletal muscle cell cultures. Preliminary experiments suggest that ELISA techniques can be used to detect circulating anodic antigen from patients with schistosomiasis. The Dot-ELISA and the ELISA were compared with the indirect fluorescent antibody test (IFAT) for detection of IgM and IgG specific antibodies to Toxoplasma. The Dot-ELISA correlated well with the IFAT (corr. coef. 0.895) and the ELISA correlated equally well with the IFAT (corr. coef. 0.910) for detecting IgG antibodies to T. gondii.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00108-14 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Studies on the Biology and Immunogenicity of Malaria Sporozoites

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | R. W. Gwadz | Senior Scientist | LPD, NIAID |
| | L. H. Miller | Section Head | LPD, NIAID |
| Others: | T. McCutchan | Research Biologist | LPD, NIAID |
| | I. Quakyi | Guest Worker | LPD, NIAID |

COOPERATING UNITS (*if any*)

New York University, School of Medicine (R. Nussenzweig, A. Cochrane, E. Enea)
WRAIR (W. Hockmeyer)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 0.4 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Circumsporozoite (CS) proteins have been shown to act as the protective antigens capable of inducing sporozoite - specific immunity in several species of malaria. The gene coding for this antigen in *Plasmodium falciparum* has been isolated, cloned and incorporated into *E. coli*. The recombinant protein induces antibodies in mice which react with sporozoites and blocks liver invasion *in vitro*.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00161-08 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunochemistry of Parasitic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | T. E. Nash | Senior Scientist | LPD, NIAID |
| Others: | L. S. Diamond | Section Head | LPD, NIAID |
| | M. N. Lunde | Research Zoologist | LPD, NIAID |
| | D. B. Keister | Biologist | LPD, NIAID |
| | A. W. Cheever | Assistant Chief | LPD, NIAID |
| | B. Ungar | Medical Staff Fellow | LPD, NIAID |
| | J. A. Dvorak | Research Microbiologist | LPD, NIAID |
| | A. Aggarwal | Visiting Fellow | LPD, NIAID |

COOPERATING UNITS *(if any)*
Animal Parasitology Institute, Beltsville, Md. (R. Fayer), Michigan State University (J. Bennett), University of Khartoum (M. Homeida).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.9 | 2.9 | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies are being performed on three parasitic parasites: schistosomes, Giardia, and Cryptosporidium. Nine more isolates of Giardia have been axenized and their DNA, surface antigens, and biologic behavior are being studied. Although still incomplete, these studies, thus far, confirm the large heterogeneity noted among the previously studied 15 isolates. Monoclonal antibodies to a 170,000 Kd major surface antigen of WB and WB-like isolates were produced. These monoclonal antibodies are specific for the WB-type of isolate and are cytotoxic to these organisms. In order to study biological importance and differences between isolates, a model infection of Giardia in jirds was established. Isolates differ in ability to invoke self cure, immunity and protection to challenge with heterologous isolates. An ELISA assay to measure antibody to Cryto-sporidium antibodies to oocysts was developed. IgG and/or IgM antibody responses in non-AIDS patients were practically always detected. IgG antibodies were found commonly in AIDS patients. In addition, IgG antibodies to Cryptosporidium were detected frequently in patients with other parasitic diseases suggesting that infection is common. A study evaluating the use of ultrasonography in the diagnosis of Symmers' fibrosis in on-going in Khartoum, Sudan.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00162-09 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | D. M. Dwyer | Supervisory Microbiologist | LPD, NIAID |
| Others: | P. A. Bates | Visiting Fellow, FIC | LPD, NIAID |
| | K. A. Joiner | Senior Investigator | LCI, NIAID |
| | K. B. Pastakia | Senior Staff Fellow | LPD, NIAID |
| | S. M. Puentes | Clinical Fellow | LCI, NIAID |
| | M. K. Wassef | Research Chemist | DHVD, NHLBI |
| | D. Zilberstein | Visiting Fellow, FIC | LPD, NIAID |
| | M. K. Kurtz | Biologist | LPD, NIAID |

COOPERATING UNITS (if any)
Dept. of Immunol. & Infect. Dis., Johns Hopkins Univ. (M. Gottlieb); Naval Biosciences Lab., Sch. Pub. Hlth., Univ. California, Berkeley (H.W. Sheppard)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Cell Biology & Immunology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.5 | 4.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The cell biology and biochemistry of Leishmania and Trypanosoma are investigated as models of intra- and extracellular parasitism, respectively. As all interactions between host and parasite occur at the level of the parasite surface membrane (SM), emphasis is placed on: 1) its integrated biochemical characterization and 2) defining its roles in parasite survival.

Using Western blots, the major SM antigens of L. donovani to which visceral leishmaniasis patients make IgG responses were identified. The glycolipid constitutents of L. donovani promastigote SM were delineated. A secreted promastigote acid phosphatase was purified, chemically characterized and monoclonal antibodies raised against it. Amastigotes also release this enzyme and visceral patients make IgG antibodies against it. Log-phase promastigotes were shown to activate and be killed by the alternate complement pathway and the primary target of C3-binding was identified as a SM protein of 140 Kd. An active transport system for ribose was demonstrated and characterized in L. donovani promastigotes. Regulation of SM 3'-nucleotidase activity was shown to be under inducible genetic control. A SM proton-ATPase which drives membrane transport processes was identified, cytochemically localized and enzymatically characterized. The SM glucose transport protein of promastigotes was identified via photoaffinity labeling and partially characterized. Genomic clones coding for parasite SM antigens were identified, isolated and characterized from an expression-vector system using both anti-SM sera and sera from visceral leishmaniasis patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00197-06 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immune Recognition in Filariasis and Other Helminth Infections

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | R. Hussain | Senior Staff Fellow | LPD, NIAID |
| Others: | E. A. Ottesen | Senior Investigator | LPD, NIAID |
| | T. E. Nutman | Medical Staff Fellow | LPD, NIAID |
| | R. B. Lal | Post-Doctoral Fellow | LPD, NIAID |

COOPERATING UNITS *(if any)*
University of Berne, Switzerland, Center for Disease Control, Atlanta, GA

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.75 | 1.0 | 0.75 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major aim of this project is to characterize immunoglobulin responses in helminth infections (primarily filariasis and schistosomiasis) with emphasis on IgE production, regulation and modulation. Sensitive radioimmunoassays have been developed and utilized for quantitating IgG and IgE antibodies. Qualitative characterization in terms of what antigens are being recognized in various clinical forms of the disease is being carried out to understand immune recognition and its implication in the pathogenesis and/or defense of the disease. These studies would in addition provide information about antigens with better specificity in immunodiagnosis or epidemiologic studies. Finally, in vitro production and regulation of IgE synthesis is also under investigation to better understand the control mechanisms of IgE production.



PHS 6040 (Rev. 1/84) GPO 914-918

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00208-05 LPD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | T. McCutchan | Senior Staff Fellow | LPD, NIAID |
| Others: | J. Mullins | Biologist | LPD, NIAID |
| | K. Vernick | Staff Fellow | LPD, NIAID |
| | J. Hansen | Staff Fellow | LPD, NIAID |
| | A. Lal | Staff Fellow | LPD, NIAID |
| | L. McNicol | Staff Fellow | LPD, NIAID |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.5 | 4.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a method for cutting intact genes out of Plasmodium DNA with mung bean nuclease. The intact genes are then ligated into an expression vector in order to make a recombinant gene bank containing genes from all stages of the parasites life cycle. We have also analyzed what types of DNA structures are involved with mung bean nuclease cleavage and modified the reaction conditions used so that this procedure works with the DNA of many higher eucaryote. From gene banks produced in this fashion we have isolated 1) a gene whose sequence has been useful in the preparation of a trial vaccine for malaria, the circumsporozoite gene on P. falciparum, 2) genes that are specifically produced in the sexual stages of P. falciparum, and 3) a gene that is involved in pyrimethamine resistance.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00240-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Culture, Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. Carter | Visiting Scientist | LPD, NIAID |
| Others: | N. Kumar | Burroughs Wellcome Senior Research Fellow | LPD, NIAID |
| | L. A. McNicol | W.H.O. Fellow | LPD, NIAID |
| | T. McCutchan | Staff Fellow | LPD, NIAID |
| | L. H. Miller | Section Head | LPD, NIAID |
| | I. A. Quakyi | W.H.O. Fellow | LPD, NIAID |
| | D. Walliker | Guest Researcher | LPD, NIAID |

COOPERATING UNITS (if any) U. of Colombo, Sri Lanka (K. Mendis); Harvard Sch. of Pub. Hlth., Boston, MA (D. Wirth); Pasteur Inst. Paris (P. David); Papua New Guinea, Inst. of Med. Res., Madang, Papua New Guinea (P. Graves); Naval Med. Res. Inst., Bethesda, MD (A. Szarfman); Case Western Res. Univ., Cleveland, OH (M. Aikawa)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.2 | 3.7 | 1.5 |

CHECK APPROPRIATE BOX(ES)
- ☐ (a) Human subjects
  - ☐ (a1) Minors
  - ☐ (a2) Interviews
- ☒ (b) Human tissues
- ☐ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The target antigens of anti-gamete transmission blocking antibodies against *P. gallinaceum* and *P. falciparum* form a complex of three proteins of approximately 250, 60 and 55 Kda in the malarial gamete membrane. The 60 and 55 Kda proteins are hydrophobic and probably embedded in the membrane; the 250 Kda protein is hydrophilic. In *P. gallinaceum* the transmission blocking Mabs recognize only the 250 Kda protein in *P. falciparum* the Mabs recognize only the 60 and 55 Kda proteins. The target epitopes appears to be destroyed by reduction.

Recombinant clones of Lambda gt-11 containing *P. falciparum* DNA fragments from a mung bean nuclease digest have been isolated. One clone may contain DNA coding the 260 Kda protein, another the 60 and/or 55 Kda protein; a third such clone expresses a fusion protein which cross reacts with antibodies against ookinetes of *P. gallinaceum* .

Isolate specific antigenic variation for the target epitope of one transmission blocking Mab has been confirmed in *P. falciparum*. Isolate specific differences in infectivity to different Anopheline species have also been demonstrated. Genetically distinct clones of *P. falciparum* are being hybridized in genetic recombination experiments during transmission through mosquitoes and primates.

Malaria transmission studies are being conducted in Papua New Guinea and in Sri Lanka. The studies in Sri Lanka have shown that transmission blocking immunity is naturally induced during acute infections of *P. vivax*.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00241-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | L. H. Miller | Section Head | LPD, NIAID |
| Others: | F. Klotz | Staff Fellow | LPD, NIAID |
| | R. Howard | Expert Consultant | LPD, NIAID |
| | D. Hudson | Biologist | LPD, NIAID |
| | M. McGinnis | Research Biologist | BB, CC |

COOPERATING UNITS (if any)
WRAIR, Washington, D.C., (T. Hadley); Hazelton Laboratories, Vienna, Va. (J. Rener); Case Western Reserve University, Cleveland, OH (M. Aikawa); CDC, Atlanta, Ga. (G. Campbell); Guys Hospital, London (G. Mitchell)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 1.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage against which immunity may work to block invasion. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as antigens for induction of protective immunity. We are now studying one antigen on the merozoite surface that undergoes antigenic variation to understand the molecular basis for this variation.

We are producing *P. falciparum* mutants that use an alternate receptor for invasion of human red cells and continuing the study of the Duffy blood group molecule that is the receptor for *P. vivax* invasion.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00242-04 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. Howard | Expert Consultant | LPD, NIAID |
| Others: | L. H. Miller | Section Head | LPD, NIAID |
| | J. A. Sherwood | Clinical Associate | LPD, NIAID |
| | A. J. Saul | Visiting Associate | LPD, NIAID |
| | L. J. Panton | Guest Worker | LPD, NIAID |
| | T. E. Wellems | Clinical Associate | LPD, NIAID |
| | W. Daniel | Research Associate | LPD, NIAID |

(see attached page)

COOPERATING UNITS (if any)

(see attached page)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.5 | 5.25 | 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


We are studying the structure and function of malarial proteins inserted into the membrane of erythrocytes infected with mature asexual malaria parasites and molecules on the endothelial cells involved in cytoadherence.

The very large cell surface protein (Mr300,000) on *P. falciparum* infected erythrocytes from *Aotus* monkeys has now been shown to be present on K+ infected cells but not K- (*i.e.* non-cytoadherent ) cells. We have also identified this molecule on infected cells directly from Gambian malaria patients. Current experiments aim to obtain the sequence of this protein in order to more precisely study its role in cytoadherence.

*P. falciparum* infected erythrocytes from Gambian patients have been shown by serological tests with antibodies from the same patients to express an extreme degree of antigenic diversity. Ten samples from children expressed ten different phenotypes of the infected cell surface antigen(s). Importantly, sera from Gambian adults contain antibodies which recognize an antigenically conserved epitope expressed by the malaria parasite on infected erythrocytes from all patients.

Thrombospondin, a protein generally associated with agglutination of activated platelets, has been identified as a potential ligand for recognition by *P. falciparum*-infected cells in the endothelial cell cytoadherence phenomenon. This protein is synthesized by endothelial cells and, as a pure protein coated on plastic or glass, will specifically bind K+ *P. falciparum*-infected cells but not K- cells. We are attempting to identify the nature of the surface component on infected erythrocytes which binds to thrombospondin.




PHS 6040 (Rev. 1/84) GPO 914-918

OTHERS

| | | |
|---|---|---|
| V. Kao | Technician | LPD, NIAID |
| S. B. Aley | Departed | (Nov. 1984) |

COOPERATING UNITS
Case Western Reserve Univ., Cleveland, OH (M. Aikawa, S. Uni); Immunology Dept. WRAIR, Washington, DC (J. Lyon); Med. Res. Council Labs., Fajara, The Gambia (K. Marsh); LSB, NIADDK (D. D. Roberts, S. J. Spitalnik, V. Ginsburg); LC, NIAMD (K. Kirk, L. Cohen); Georgetown Univ., Washington, DC (D. Taylor); Christian Albrecht's Univ., Kiel, W. Germany (R. Schauer)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00244-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Developmental Adaptations of Trypanosoma cruzi to the Vertebrate Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | A. Sher | Section Head | LPD, NIAID |
| Others: | L. V. Kirchhoff | Medical Staff Fellow | LPD, NIAID |
| | K. Joiner | Senior Investigator | LCI, NIAID |
| | D. Sacks | Senior, Staff Fellow | LPD, NIAID |

COOPERATING UNITS (if any)
Wellcome Biotechnology LTD, Kent, England (D. Snary)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.4 | 1.2 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In this project, we have been studying developmental adaptations of Trypanosoma cruzi to the vertebrate host, and in particular, surface membrane changes occuring during the morphogenesis of epimastigotes (vector stage) to metacyclic trypomastigotes (vertebrate stage). During the report period, we continued to investigate the mechanism by which metacyclic trypomastigotes become resistant to lysis by the alternative pathway of complement (ACP). Studies with purified complement components revealed that in comparison to epimastigotes, metacyclic trypomastigotes bind minimal amounts of Factor B, an essential co-factor in the ACP. In contrast, to the findings obtained with other systems, binding of the regulatory component H was equivalent in activating epimastigotes and non-activating metacyclic trypomastigotes. Enzyme treatment studies suggested that the incapacity of metacyclic trypomastigotes to activate the ACP was due to their elaboration of pronase and tunicamycin sensitive molecules.

In related studies, we investigated the expression of a 72,000 MN glycoprotein (GP72) (previously shown by us to be the acceptor for C3 on epimastigotes) on insect and culture derived metacyclic trypomastigotes. Using monoclonal antibodies, GP72 was detected on the surface of insect metacyclics but was not found on culture generated organisms. Since by surface labelling and immunoprecipitation we were able to detect the molecule on this stage, it apparently is modified and expresses fewer epitopes accessible to antibody binding on intact parasites than its equivalent on epimastigotes.

Finally, using rabbit anti-idiotypic antibodies directed against an anti-GP72 monoclonal antibody, we were able to raise an antibody response against carbohydrate determinants on the GP72 molecule in mice, rabbits and guinea pigs.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00246-03 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | A. Sher | Section Head | LPD, NIAID |
| Others: | E. J. Pearce | Fogarty Fellow | LPD, NIAID |
| | D. Lanar | Guest Worker | LPD, NIAID |
| | T. McCutchan | Senior Investigator | LPD, NIAID |

COOPERATING UNITS (if any)
Biomedical Research Institute, Rockville, MD

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.7 | 1.5 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our progress this year consisted of a study of the dynamics of schistosome surface antigen expression in vivo and the cloning in an expression vector of several S. mansoni genes encoding antigens recognized by immune mice.

A. Surface antigen modulation. While in vivo derived schistosomula express little if any serologically detectable surface antigen, schistosomula maturing in vitro retain their surface antigenicity. This difference was investigated and shown to be due to the rapid shedding of surface antigens when schistosomula are transferred from the in vitro to the in vivo environment.

B. Antigen cloning studies. From a series of clones selected from a lambda gt-11 S. mansoni library, two clones were identified which encode antigens recognized by immune mouse antibodies. The antigenicity and immunogenecity of the fusion proteins produced by these clones were characterized.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00248-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Genetics and Physiology of Vector Capacity in Anopheline Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. W. Gwadz | Senior Scientist | LPD, NIAID |
| | L. H. Miller | Section Head | LPD, NIAID |
| | R. K. Sakai | Expert | LPD, NIAID |
| Others: | R. Galun | Visiting Scientist | LPD, NIAID |
| | I. A. Quakyi | Guest Worker | LPD, NIAID |
| | P. A. J. Romans | Guest Worker | LPD, NIAID |
| | K. D. Vernick | Bio. Lab. Tech. | LPD, NIAID |
| | H. G. Coon | Senior Investigator | LG, NCI |

COOPERATING UNITS (if any)

Red Cross Blood Center, Bethesda, MD (R. Williams and T. Takahashi)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Malaria

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.5 | 3.8 | 2.7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are attempting to develop systems for <u>cloning</u> and introducing <u>genes</u> into <u>mosquito germ lines</u>. In addition we are attempting to define factors which render <u>mosquitoes refractory</u> to <u>malarial infection</u>. Special emphasis is being placed on the genetics of <u>Anopheles gambiae</u> as it relates to <u>Plasmodium falciparum</u>, the primary vector/parasite combination in sub-Saharan Africa. Concurrently, a simian malaria, <u>P</u>. <u>cynomolgi</u> is being used as a model for these studies. Mode of inheritance and the <u>physiological basis for refractories</u> is being studied in one selected strain of <u>An</u>. <u>gambiae</u>.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00251-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | A. Sher | Section Head | LPD, NIAID |
| Others: | E. J. Pearce | Fogarty Fellow | LPD, NIAID |
| | R. C. Oliveira | Guest Worker | LPD, NIAID |
| | D. Zilberstein | Guest Worker | LPD, NIAID |

COOPERATING UNITS (if any)
George Washington University (S.L. James), University of North Carolina (D. Mcall), Montreal General Hospital (E. Skamene)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.3 | 2.5 | 0.8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The aim of this project is to study mechanisms of immunity and immune evasion in schistosomiasis with the ultimate goal of developing an experimental vaccine employing defined antigens:

A. Genetics of protective immunity in the irradiated vaccine model. A single gene defect was identified by means of genetic crosses which controls the deficient irradiated vaccine induced immunity of A/J mice. Using recombinant inbred strains the gene was tentatively mapped to chromosome 6 of the mouse. Genetic complementation experiments revealed that the gene locus controlling the A/J mouse immune defect is distinct from that controlling the deficient immunity of P mice.

B. Mechanisms of immune evasion of developing schistosomula. Schistosomula developing in vitro were shown to become resistant to killing by activated macrophages. This phenomenon was temporally correllated with a change in membrane permeability to the probe tetraphenylphosphonium. Susceptibility to activated macrophage killing was shown to be regained in 2½ week old worms but lost in older parasites suggesting that 2½ week old schistosomes may be a target for late stage immunity.

C. Identification of vaccine immunogen. A 97 Kd soluble schistosome protein was identified as the sole antigen recognized by antibodies from mice vaccinated with BCG plus schistosome extracts. Antigen purification and vaccination experiments supported the hypothesis that this molecule is the immunogen responsible for the induction of protective immunity in the BCG model.




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00253-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Immunologic Responses to Filarial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | E. A. Ottesen | Senior Investigator | LPD, NIAID |
| Others: | R. Hussain | Senior Staff Fellow | LPD, NIAID |
| | T. B. Nutman | Medical Staff Fellow | LPD, NIAID |
| | D. Ward | Medical Staff Fellow | LPD, NIAID |
| | R. Davey | Medical Staff Fellow | LCI, NIAID |
| | R. Lal | Visiting Fellow | LPD, NIAID |
| | M. N. Lunde | Research Zoologist | LPD, NIAID |

COOPERATING UNITS (if any)
Indian Council of Medical Research Tuberculosis Research Centre, Madras India (S. P. Tripathy, R. Prabhakar, P. R. Narayanan, V. Kumaraswami, R. Paranjape and V. Vijayan); Onchocerciasis Chemotherapeutic Research Centre, Tamale, Ghana

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.8 | 4.8 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with filariasis.

Qualitative analysis of filaria-specific IgE and IgG subclass antibodies indicates different antigen recognition patterns among groups of patients with different clinical manifestations of filariasis and a special "linkage" or parallelism between the recognition patterns for IgE and $IgG_4$ antibodies. This latter may account for the finding of "blocking antibodies" that control immediate hypersensitivity responses in patients with this and other chronic helmith infections.

Immunoregulatory studies have extended the description of immune suppression in microfilaremic patients to include antibody production as well as T-cell induced blastogenesis. The lymphokines IL-2 and Gamma-interferon are not produced by the cells of such patients if stimulated in vitro by filarial antigen but are induced by the presence of other non-filarial antigens or mitogens.

Assessment of local pulmonary pathology in patients with tropical eosinophilia (TPE) by bronchoalveolar lavage (BAL) has indicated extreme elevations of specific IgE, IgG and IgM antibodies, increased cell numbers and abnormal production of oxidants by alveolar cells. Longitudinal BAL studies show that such alveolitis persists in patients despite standard treatment regimens and, therefore, suggest that more vigorous therapy for TPE must be developed.




PHS 6040 (Rev. 1/84)

GPO 914-918

PRINCIPAL INVESTIGATOR continued:

| | | | |
|---|---|---|---|
| Others: | D. Volkman | Senior Investigator | LIR, NIAID |
| | H. Francis | Senior Investigator | LIR, NIAID |
| | M. M. Frank | Chief | LCI, NIAID |
| | R. G. Crystal | Chief | PB, NHLBI |
| | P. Pinkston | Medical Staff Fellow | PB, NHLBI |
| | W. Rom | Visiting Associate | PB, NHLBI |
| | R. Nussenblatt | Chief | CB, NEI |
| | T. J. Lawley | Senior Investigator | DB, NCI |

COOPERATING UNITS continued:

(K. Awadzi, and D. Badu); Special Programme for Tropical Disease Research, WHO, Geneva; Centers for Disease Control (C. Reimer).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Immunologic Responses to Non-Filarial Helminth Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | E. A. Ottesen | Senior Investigator | LPD, NIAID |
| Others: | C. J. Maxwell | Medical Staff Fellow | LPD, NIAID |
| | R. Hussain | Senior Staff Fellow | LPD, NIAID |
| | T. B. Nutman | Medical Staff Fellow | LPD, NIAID |
| | J. White | Medical Staff Fellow | LCI, NIAID |
| | J. I. Gallin | Head, Cell Physiology Section | LCI, NIAID |

COOPERATING UNITS (if any)

Tulane University (B. Cline, S. Katz, and D. Little)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.1 | 1.1 | 0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)
Experimental hookworm (Necator americanus)infections in normal volunteers have been extablished with a goal toward defining progressive changes in immunologic responses (especially IgE) to helminth infection. An infecting dose of 50 filariform larvae was sufficient to stimulate vigorous eosinophil and moderate IgG and IgE antibody responses but was insufficient to induce strong or consistent lymphocyte blastogenic responses. Changes in eosinophil functional responsiveness were also noted.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00256-04 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Differentiation of Leishmania Promastigotes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | D. L. Sacks | Senior Staff Fellow | LPD, NIAID |
| Other: | F. A. Sher | Section Head | LPD, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previous studies have determined that sequential development of Leishmania promastigotes from a noninfective to an infective stage accompanies the growth of parasites both within culture and the sandfly vector. Infective or metacyclic L. major promastigotes can now be purified from culture on the basis of their loss of binding sites for the lectin peanut agglutinin (PNA). A metacyclic stage specific surface antigen (116,000 M.W.) has been detected on the basis of Western blot analysis and immunoprecipitation of surface labeled organisms. In addition, a monoclonal antibody has been produced which binds strongly to the surface of metacyclic promastigotes and only weakly to log phase parasites. The role of these surface changes in the development of promastigotes within the sandfly as well as their ability to visit the leishmanicidal activities of normal macrophages and serum are currently being studied.

The immunology of human visceral leishmaniasis is being studied in a collaborative project recently initiated by Dr. Frank Neva with the Rajendra Memorial Research Institute in Patna, India. In initial studies on 18 patients with acute visceral disease responsiveness to leishmanial antigens was found to be profoundly suppressed. Removal of OKT8+ lymphocytes did not reconstitute these responses. Kala-azar patients maintained good responses to mitogens and PPD. The immunological basis of this specific unresponsiveness will be pursued during future visits.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00257-04 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | F. A. Neva | Chief | LPD, NIAID |
| Others: | W. T. London | Chief, Sect. Expt'l. Pathology | IRP, NINCDS |
| | K. Barrett | Visiting Fellow | LCI, NIAID |
| | T. B. Nutman | Medical Staff Fellow | LPD, NIAID |
| | E. A. Ottesen | Head, Clin. Parasitol. Sect. | LPD, NIAID |
| | P. J. Brindley | Visiting Fellow | LPD, NIAID |
| | C. A. Maxwell | Medical Staff Fellow | LPD, NIAID |
| | D. Alling | Special Assist for Biometry | LCI, NIAID |

COOPERATING UNITS (*if any*)

Meloy Laboratories, Rockville, MD, (G. Phillips); Veteran's Administration Hospital, Wichita, Kansas (L. Pelletier)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.4 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


The focus of this project continues to be on certain aspects of the immune response to infection with Strongyloides stercoralis in humans as well as in the experimental host, the Patas monkey. A strong but not complete degree of protection to challenge was demonstrated in several previously infected Patas monkeys. The presence of intestinal mast cells appears to be a prominent feature of chronic strongyloides infection in the monkey. Increasing experience with the immediate skin test composed of somatic and metabolic larval antigens indicates that it is useful for diagnostic purposes in human infections. Reaction sizes for positive tests have been determined. Analysis of larval antigens using polyacrylamide gel electrophoresis and immuno-blotting has been initiated.




PHS 6040 (Rev. 1/84)

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00258-04 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Development of Models for Chagas' Disease Using *T. cruzi* Clones and Inbred Mice

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | |
|---|---|---|
| M. Postan | WHO/Visiting Associate | LPD, NIAID |
| J. A. Dvorak | Res. Microbiologist | LPD, NIAID |
| J. Bailey | Chief, Med. Application Sect. | LAS, DCRT |
| E. Pottala | Senior Engineer | LAS, DCRT |

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Inbred mice and rats and *T. cruzi* clones are being used to develop experimental models for Chagas' disease. The studies confirm the importance of both the natural heterogeneity of the parasite population in chronic chagasic patients and the parasite's role in the course and outcome of a *T. cruzi* infection. Various complex aspects of human disease can be separated using inbred rats and mice infected with different *T. cruzi* clones indicating the potential usefulness of this system in developing models for Chagas' disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00347-03 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. W. Cheever | Assistant Chief | LPD, NIAID |
| Others: | R. H. Duvall | Bio. Lab. Tech. (Micro.) | LPD, NIAID |
| | J. Malley | Mathematical Statistician | LSM, DCRT |
| | K. Malley | Computer Assistant Analysist | LSM, DCRT |
| | A. Sher | Head, Immunology Section | LPD, NIAID |

COOPERATING UNITS *(if any)*

Brigham & Women's Hospital, Boston, Mass., Dept. of Pathology (F. V. Lichtenberg, J. Byram)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 0.75 | 0.75 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatic fibrosis is examined in mice infected with schistosome species pathogenic for man. Mouse strains developed markedly different degrees of hepatic fibrosis following infection with *S. mansoni*. T cells are important for the formation of granulomas in both *S. mansoni* and *S. japonicum* infected mice, as determined from examination of athymic and B cell depleted mice. Current studies are focused on determining which subsets of T cells are involved in regulation of *S. japonicum* egg granulomas. The intensity of murine *S. japonicum* infection has been shown to have a profound effect on the regulation of hepatic pathology. More heavily infected mice have smaller circumoval granulomas and less fibrosis in relation to the number of eggs present in the liver, i.e. less fibrosis per egg.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00348-03 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunity in Murine Schistosomiasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. W. Cheever | Assistant Chief | LPD, NIAID |
| Others: | R. H. Duvall | Bio. Lab. Tech (Micro) | LPD, NIAID |
| | A. Sher | Section Head | LPD, NIAID |
| | J. Malley | Mathematical Statistician | LSM, DCRT |
| | K. Malley | Computer Assistant Analyst | LSM, DCRT |
| | P. Shade | Bio. Lab. Tech. | LPD, NIAID |

COOPERATING UNITS *(if any)*
Biomedical Research Institute, Rockville, MD (F. Lewis and C. Richards); George Washington University (S. James)

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host-Parasite Relations Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | 0.25 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Substrains of S. mansoni selected for varying infectivity to vector snails have been tested for their ability to induce immunity in mice. Two strains derived from the same patient and designated PRT-3 and PRC-3 induced markedly different degrees of resistance to reinfection after a bisexual first infection. The F-1 cross between these strains also produced high resistance, comparable to that induced by the "immunogenic" PRT-3 strain. C57BL/KsJ mice with unisexual S. mansoni infections generally were about 40% resistant to challenge infections; however, the PRC-3 strain induced no resistance after unisexual infection. The PRT-3 and PRC-3 strains have been selected for 5 generations on the basis of their ability to induce resistance, but no apparent selection of higher or lower resistance traits has been achieved.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00350-03 LPD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | T. E. Nash | Medical Officer | LPD, NIAID |
| Others: | D. B. Keister | Biologist | LPD, NIAID |
| | J. A. Dvorak | Research Microbiologist | LPD, NIAID |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nine new isolates of Giardia have been axenized and are in the process of being studied.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00351-03 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | P. A. Scott | Staff Fellow | LPD, NIAID |
| Others: | A. Sher | Section Head | LPD, NIAID |
| | D. Sacks | Staff Fellow | LPD, NIAID |
| | F. A. Neva | Chief | LPD, NIAID |
| | P. Natovitz | Biologist | LPD, NIAID |
| | D. Dwyer | Supvr. Microbiologist | LPD, NIAID |

COOPERATING UNITS *(if any)*
Wellcome Research Laboratories, Experimental Biology Div., London, England (J. Howard); George Washington University, Washington, D.C. (S. James).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Immunology and Cell Biology

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 1 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Leishmaniasis is a chronic protozoal disease of man. Our goal is to define mechanisms responsible for this chronicity using experimental murine infections. We described several factors that influence cutaneous leishmaniasis, including: (1) resistance to killing of some leishmanial strains, (2) impaired ability of macrophages to be activated to kill Leishmania at cutaneous temperatures, and (3) inability of specifically elicited macrophages from infected BALB/c mice to kill Leishmania. We found that infected BALB/c mice also develop a peritoneal eosinophilia, and the relationship between this eosinophilia and BALB/c susceptibility is being investigated. In addition, Mu-suppression from birth altered leishmanial infections in C3H/HeN mice, converting a self-healing infection to a chronic one. Results from cell transfer experiments suggest that B cells and/or antibodies may be required for the development of a T cell necessary for healing leishmanial infections. Finally, we used an experimental vaccine to study how the chronicity of leishmanial infections can be circumvented. Immunization of C3H/HeN mice was achieved by intradermal injection of irradiated promastigotes, while protection in BALB/c mice was only obtained by the intravenous route. At present, the effector mechanism of this vaccine is not known. However, we found that macrophages from protected mice do not develop the resistance to activation described above. With regard to the immunogens involved, both particulate and soluble fractions were protective, but only if they were isolated with the appropriate protease inhibitors. Moreover, preliminary experiments suggest that while logarithmic growth phase promastigotes and amastigotes are protective, stationary growth phase promastigotes are not protective. Work is currently focused on defining the protective immunogens.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00439-01 LPD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical and Therapeutic Studies in Human Filariasis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | E. A. Ottesen | Senior Investigator | LPD, NIAID |
| Others: | T. B. Nutman | Medical Staff Fellow | LPD, NIAID |
| | D. Ward | Medical Staff Fellow | LPD, NIAID |
| | R. Davey | Medical Staff Fellow | LCI, NIAID |
| | R. G. Crystal | Chief | PB, NHLBI |

COOPERATING UNITS *(if any)* Tuberculosis Research Centre, Madras, India (Dr. R. Prabhakar, and Dr. V. Kumaraswami); Madras Medical College, Madras, India (Dr. K. Vijayasekaran); Peace Corps Medical Office (Dr. K. D. Miller and Dr. N. Reinhart).

LAB/BRANCH
Laboratory of Parasitic Diseases

SECTION
Host Parasite Relations

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .5 | .5 | 0 |

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither
[ ] (a1) Minors
[X] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Trials of two new drugs, invermectin (Phase IIa) and CGP-20376 (Phase I), are being initiated in collaboration with investigators from Madras India for patients with bancroftian filariasis.

Since bronchoalveolar lavage studies on patients with tropical pulmonary eosinophilia show persistent alveolitis despite standard therapy with diethylcarbamazine (DEC), a trial comparing long-term DEC or DEC plus steroids with standard therapy has commenced.

Clinical evaluation of Peace Corps Volunteers working in Central/West Africa indicates that when such expatriates enter Loa loa endemic areas, they become immunologically hypersensitive to the parasite and often develop severe clinical symptoms. To develop means for protecting such individuals from infection, a double-blind trial of prophylactic weekly DEC is now in its second year.




PHS 6040 (Rev. 1/84) GPO 914-918

LABORATORY OF VIRAL DISEASES
1985 ANNUAL REPORT
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
October 1, 1984 to September 30, 1985

Dr. Bernard Moss
Chief, Laboratory of Viral Diseases

The Laboratory of Viral Diseases carries out a program of fundamental investigations on the molecular biology of viruses, the interactions of viruses with host cells, the pathogenesis of viral diseases, and host defense mechanisms. Current topics of research include: regulation of gene expression, mechanisms of DNA replication, virus growth factors, development of recombinant expression vectors, factors determining virus virulence, host resistance genes, targets of cell-mediated immunity, and genetic engineering of live recombinant vaccines.

Highlights of this year's research efforts are summarized below.

Regulation of Vaccinia Virus Gene Expression

Vaccinia virus has a genome of 185,000 bp that encodes approximately 200 polypeptides. These genes are expressed in a coordinated fashion so that some polypeptides are made before and others after DNA replication. One vaccinia virus gene that is expressed throughout the growth cycle was found to have two RNA start sites about 55 bp apart. The site nearest to the coding segment was used at early times in infection and the other was used at late times. *In vitro* mutagenesis studies revealed that there are two independent promoters and that the regulatory signals are located within 31 bp of each RNA start site (Cochran and Moss).

RNA polymerase subunits, synthesized in reticulocyte lysates programmed with early vaccinia virus mRNA, were immunoprecipitated by antibody prepared against the purified enzyme. The subunit genes were mapped by hybridization selection of mRNA to cloned DNA fragments prior to translation. The genes for 2 subunits of 147 kD and 22 kD were sequenced. The large subunit was shown to have considerable homology with the β' subunit of *E. coli* RNA polymerase (Broyles and Moss).

A soluble extract capable of selectively transcribing added early vaccinia virus genes was prepared by disrupting purified vaccinia virus particles. Correct initiation, termination and polyadenylation were demonstrated and the signals for each were defined by transcription of truncated templates. Termination was shown to occur about 50 bp downstream of the signal (Rohrmann, Yuen and Moss).

Regulation of Expression of Herpes Simplex Virus Genes.

The expression of the HSV-1 glycoprotein C gene was studied by ligating the promoter for this gene to the coding sequence for the

bacterial enzyme, β-galactosidase. Mammalian cells that were transfected with this recombinant plasmid expressed β-galactosidase only when the cells also were infected with HSV-1 or were co-transfected with a plasmid recombinant containing two of the HSV-1 immediate-early genes. This transient assay system will be useful for determining the sequences in the glycoprotein C promoter that are important for regulation (Weir).

Structure and Replication of Poxvirus DNA

Poxviruses provide a unique experimental system for studying DNA replication. The ends of the linear double-stranded DNA genome consist of hair-pin structures that may resemble telomeres of eukaryotic chromosomes. Enzymes and other proteins needed for DNA synthesis are encoded within the viral genome and replication occurs in the cytoplasmic compartment of infected cells. During the past year the DNA polymerase gene of vaccinia virus was completely sequenced and the primary structure of the enzyme was derived. A computer search of the National Biomedical Research Foundation protein data base revealed a significant homology between the vaccinia DNA polymerase and the DNA polymerases of Epstein-Barr virus and adenovirus. These data suggest that the DNA polymerases of poxviruses, herpesviruses and adenoviruses are related (Earl and Moss).

Previous studies from this laboratory demonstrated the presence of concatemeric forms of vaccinia virus DNA in infected cells. The junction region, between individual genome units in these concatemers, was stably cloned in an Escherichia coli plasmid. Restriction endonuclease analysis indicated that the junction consists of an imperfect palindrome. The nucleotide sequence of the complementary strands was identical to that of the flip-flop hairpin loops at the ends of mature genomes. In cells that were infected with vaccinia virus, plasmids containing 250 bp or more of the junction were replicated and converted into minichromosomes with vaccinia DNA hairpins at each end and plasmid DNA in the center. An enzyme that carried out similar or identical cleavage and ligation reactions was isolated and is being characterized (Merchlinsky and Moss).

Vaccinia virus growth in BSC-1 or HeLa cells was inhibited by aphidicolin concentrations greater than 20 μM. After treatment of the wild-type virus with hydroxylamine, a viral mutant was isolated which is resistant to 80 μM aphidicolin. In an in vitro assay, viral DNA polymerase isolated from cells infected with mutant virus was more resistant to aphidicolin that viral DNA polymerase isolated from cells infected with wild-type virus. Transfer of aphidicolin-resistance was achieved by transfecting cells infected with wild-type virus with DNA isolated from aphidicolin-resistant virus. Production of aphidicolin-resistant recombinant viruses was measured by plaque assay in the presence of aphidicolin. The site of the mutation was located within a 194 bp fragment of the DNA polymerase gene (DeFilippes).

## Replication of Adenovirus DNA

We have previously shown that the origin of adenovirus DNA replication is comprised of two functionally distinct domains: a ten base pair sequence which probably represents the binding site for a viral initiation protein and an adjacent 20 base pairs which constitutes the binding site for a cellular protein, Nuclear Factor I. We have concentrated in particular on the Nuclear Factor I binding site. Using oligonucleotide mutagenesis we have constructed plasmids with point mutations in the binding site region. Studies with these mutants both _in vitro_ and _in vivo_ have established the following conclusions: 1) the nucleotide sequence specifically recognized by Nuclear Factor I is TGG(N6)GCCAA; 2) the Nuclear Factor I binding site is required for replication _in vivo_ as well as _in vitro_ 3) tight binding of Nuclear Factor I to the origin is a necessary, but not sufficient condition for initiation _in vitro_ (Challberg).

## Vaccinia Virus Growth Factor

The recent discovery, that a vaccinia virus gene encodes a polypeptide with structural homology to transforming growth factor (TGF-α) and epidermal growth factor (EGF), led us to look for a virus-induced protein with the predicted biological activity. The supernatant of infected cell cultures were found to contain an acid stable $M_r$ 25,000 polypeptide which competes with EGF for binding to EGF membrane receptors. This vaccinia virus-induced growth factor (VGF) like EGF and TGF-α is mitogenic and stimulates anchorage independent cell growth in the presence of TGF-β. However, VGF did not cross-react in a radioimmunoassay specific for small and large forms of TGF-α and exhibited minimal cross-reactivity with antisera to EGF. VGF was detectable in the culture medium within 2 hr and maximal amounts were present 12 hr after infection. The level of VGF was proportional to the multiplicity of vaccinia virus used. Inhibition of viral DNA synthesis enhanced VGF production, consistent with the hypothesis that VGF is an early viral gene product. The demonstration of a novel growth factor, that is released from cells infected with vaccinia virus, may have important implications regarding virus-host interactions. In order to determine the nature of these interactions, a vaccinia mutant that does not produce VGF was isolated and is being studied (Chakrabarti and Moss).

## Development of Vaccinia Virus as an Expression Vector

Procedures have been developed for the use of vaccinia virus as a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. To facilitate the formation and isolation of recombinant virus, new plasmid vectors have been constructed which direct the insertion of the chimeric gene together with the _E. coli_ β-galactosidase gene into the thymidine kinase locus. Recombinant virus is then selected on the basis of the thymidine kinase negative phenotype and/or staining with a β-galactosidase indicator dye. The recombinant viruses produced in this manner are stable and have a wide

host range for tissue culture cells and animals (Chakrabarti and Moss).

Expression of Herpes Simplex Virus Type-1 Glycoprotein D.

Infectious vaccinia virus recombinants that contain the herpes simplex virus type 1 (HSV-1) glycoprotein D (gD) gene under control of defined early or late vaccinia virus promoters were constructed. Tissue culture cells infected with these recombinant viruses synthesized a glycosylated protein that had the same molecular weight as the gD protein produced by HSV-1. Immunization of mice with one of these recombinant viruses by intradermal, subcutaneous or intraperitoneal routes resulted in production of antibodies that neutralized HSV-1 and protected mice against subsequent lethal challenge with HSV-1 or HSV-2. Immunization with the recombinant virus also protected the majority of the mice against the development of a latent HSV-1 infection of the trigeminal ganglia. This is the first demonstration that a genetically engineered vaccine can prevent the development of latency (Moss).

Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants.

Development of effective vaccines against respiratory viruses requires a thorough understanding of the immunological response to individual proteins. Recombinant vaccinia viruses have been constructed that express individual influenza virus genes. Tissue culture cells infected by vaccinia recombinants expressing heamagglutinin (HA-VAC) or nucleoprotein (NP-VAC) synthesized authentic influenza polypeptides which were expressed on the cell surface and which were recognized by anti-influenza A virus cytotoxic T lymphocytes (CTL) in a H-2 restricted manner. Animals vaccinated with HA-VAC recombinants produced antibodies against HA (anti-HA) and were protected against subsequent intranasal challenge with influenza virus of the homologous subtype. Mice immunized with HA-VAC or NP-VAC were primed for secondary anti-HA or anti-NP CTL responses, respectively. Anti-HA CTL were mostly subtype specific while anti-NP CTL were strongly crossreactive among all influenza A virus subtypes (Smith and Moss).

A cDNA copy of the glycoprotein G gene of respiratory syncytial virus has been inserted into the vaccinia virus genome. A glycosylated protein of 84 kD was synthesized and immunoprecipitated with specific antiserum. Transport to the cell surface was demonstrated by immunofluorescence (Elango and Moss).

Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants

Hepatitis is a serious world-wide health problem. Approximately 200 million people are chronically infected with hepatitis B virus and large numbers of deaths are attributed to fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. Although an effective subunit vaccine has been produced, limitations in supply and expense have prevented its global use. As an alternative, we are trying to construct a live recombinant hepatitis B vaccine. The gene for

hepatitis B virus surface antigen has been engineered and inserted into the genome of vaccinia virus. The recombinant vaccinia virus is stable and expresses high levels of the hepatitis virus protein. Vaccination of chimpanzees resulted in priming of the immune system and protection against clinical hepatitis upon subsequent challenge with hepatitis B virus. Vaccinia virus recombinants that express higher levels of HBsAg have been constructed by using the promoter from a major structural protein of vaccinia virus and are being evaluated (Brechling and Moss).

Recent studies have indicated that the DNA sequence preceding the HBsAg gene, referred to as pre-S, is expressed by hepatitis B virus and contains immunologically dominant epitopes. A vaccinia virus recombinant that express the entire long open-reading-frame was constructed. Rabbits vaccinated with this recombinant produced antibodies to pre-S and S epitopes (Cheng and Moss).

Expression of the Rabies Virus Glycoprotein Gene by Vaccinia Virus Recombinants.

Vaccinia virus recombinants that contain the rabies glycoprotein gene under control of the vaccinia virus 11K promoter were constructed. Synthesis and correct processing was demonstrated by immunoprecipitation and immunofluoresence studies. Vaccinated mice produced antibodies to the rabies glycoprotein and were protected against intracerebral infection with rabies virus (Brechling and Moss).

Expression of Malaria Genes by Vaccinia Virus Recombinants

Malaria remains a serious global health problem for which there is no effective vaccine. Previous studies indicate that animals can be immunized with inactivated sporozoites. The genes coding for the circumsporozoite (CSP) antigens of the malaria parasite _Plasmodium knowlesi_ and _P. falciparum_ were inserted into the vaccinia virus genome under the control of a defined vaccinia virus promoter. Tissue culture cells infected with the recombinant synthesized polypeptides that reacted with monoclonal antibody against the malaria protein. Studies on the sequence of the expressed _P. falciparum_ CSP indicated that the $NH_2$-terminus is blocked and COOH-terminus is not processed. Immunofluorescent staining demonstrated that the CSP was distributed primarily in the cytoplasm of infected cells. Rabbits vaccinated with the recombinant virus produced antibodies that bound specifically to sporozoites (Cheng and Moss).

The S antigen gene of _P. falciparum_ also was expressed in a vaccinia virus recombinant. The protein was secreted from infected cells and reacted with specific antiserum. Vaccinated animals produced a low but detectable antibody response (Moss).

Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS.

Retroviruses, long associated with leukemia and sarcoma of animals, have recently been implicated as the etiological agents of

human T cell leukemia and human acquired immune deficiency syndrome (AIDS). The identification of these agents makes it possible to consider various ways of prevention. The most promising approach is development of a vaccine that could be administered to individuals at risk. Since human retroviruses have not yet been shown to produce disease in animals, initial vaccine work must be done with animal retroviruses. Friend leukemia virus complex is a useful model system since it produces an acute disease in adult mice which can be prevented by repeated immunization with the envelope glycoprotein. The envelope gene of Friend murine leukemia virus (MuLV) was inserted into vaccinia virus under the control of a vaccinia virus promoter that is active at early and late times after infection. Pulse-labeling experiments indicated that and MuLV polypeptide of 85 kD was synthesized and subsequently processed to polypeptides of 70 kD and 15 kD. Immunofluorescence studies indicated that the 70 kD polypeptide was inserted into the cell membrane. Mice vaccinated with the recombinant virus produced antibodies to the MuLV envelope protein and were protected against the development of splenomegaly upon intravenous challenge with MuLV (Earl and Moss).

A similar procedure was used to prepare vaccinia virus recombinants that express the envelope gene of HTLV-III, the causative agent of AIDS. Immunoblotting and immunoprecipitation studies indicated that the 160 kD polypeptide is synthesized and processed correctly. The ability of this recombinants to induce an immune response in experimental animals is under investigation (Chakrabarti and Moss).

Expression of Vesicular Stomatitis Virus (VSV) Protein.

VSV causes a contagious disease of horses, cattle and pigs. When cDNA copies of mRNAS of VSV were linked to a vaccinia virus promoter and inserted into the vaccinia genome, the recombinants retained infectivity and synthesized VSV polypeptides. The G protein was glycosylated and inserted into the plasma membrane of the cell. After intradermal vaccination with live recombinant virus expressing the G protein, mice produced VSV neutralizing antibodies and were protected against lethal encephalitis upon intravenous challenge with VSV. In cattle, the degree of protection against intradermalingually injected VSV was correlated with the level of neutralizing antibody produced following vaccination (Moss).

Association of the Influenza Hemagglutinin with Extracellular Vaccinia Virus.

The possibility has been considered that the foreign proteins expressed by vaccinia virus recombinants may become associated with vaccinia virus and alter its properties. In particular, envelope glycoproteins might become incorporated into the membranes of vaccinia virus. To evaluate this, the influenza hemagglutinin (HA) gene was transferred from the WR strain of vaccinia virus, which produces very little extracellular virus, to the IHD strain, which produces large amounts. The presence of the influenza HA on the cell surface was confirmed by immunofluorescence of infected cells. The influenza HA could be detected in association with extracellular virus released

into the medium. This was demonstrated by immunoprecipitation, immunoblotting, and hemagglutination of chicken red blood cells. Preliminary experiments indicated that treatment of the extracellular virus with influenza HA monoclonal antibodies resulted in a significant reduction of infectivity (Kotwal, Buller, and Moss).

Pathogenesis of Orthopoxvirus Infections

Ectromelia, an orthopoxvirus, causes mousepox in colonized mice. Studies were carried out: 1) to improve the surveillance and control measures necessary to prevent future mousepox epizootics in the mouse colonies; and 2) to study the molecular basis of othopoxvirus pathogenesis in inbred mice, and to apply the acquired knowledge towards the development of a safe, effective recombinant vaccinia virus vaccine for human use.

1) Mousepox control measures: These studies showed that the current practice of immunizing mice against mousepox with vaccinia virus IHD-T is of dubious value and should only be carried out in exceptional circumstances. It was also demonstrated that all cell lines (especially hybridomas) passed in mice potentially can become infected with ectromelia virus, and can result in new cases of mousepox on reinoculation into previously unexposed mice (Buller).

2) Virus pathogenesis studies: This research was expanded this year and carried out in two mutually supportive directions, firstly a number of different regions of the orthopoxvirus genome were evaluated for their contribution to virus virulence in mice and rabbits. The thymidine kinase, and vaccinia growth factor genes, as well as a 9 kb protein coding region proximal to the left hand terminus of the genome, were shown to contribute to virus virulence (Buller and Moss). The second area of investigation involved the genetic analysis of inbred mouse strains in order to determine the number and importance of non-H-2 genes responsible for recovery from mousepox. The resistance genes segregated as a multifactorial dominant locus, and a portion of the resistance phenotype appeared to be sex-linked. Subsequent work will focus on the mechanism by which these genes protect the host from mousepox (Buller).

Studies on the Treatment of Disease with the Interferon System

Clinical and preclinical observations indicate that Poly ICLC should be used at lower levels than we have been using it. The use of lower levels maximizes its immune enhancing activity; there being an optimum drug level above which there is actually decreased effectiveness and increased toxicity. It also was revealed that Poly ICLC is the most effective biological response modified that the N.C.I. has tested. Modest, but significant clinical improvement has been seen in juvenile laryngopapilloma, multiple myeloma and multiple sclerosis, but not leukemia, breast cancer or neuroblastoma at the high drug levels used (Levy).

## Administrative Changes

During the past year, the second phase in the reorganization of the Laboratory of Viral Diseases was completed. Members of the Viral Oncology Section and the Viral and Cellular Immunology Section were transferred to the newly formed Laboratory of Immunopathology. Drs. Geoffrey Smith and Elaine Jones completed their training and accepted independent positions at the University of Cambridge and Smith, Klein and French Laboratories, respectively. Dr. George Rohrmann, on leave from the University of Oregon, spent a very productive 10 month period here working on *in vitro* transcription systems. Drs. Thomas Fuerst (Staff Fellow), Charles Flexner (Medical Staff Fellow), Navayanasa Elango (Visiting Associate), Girish Kotwal (Visiting Fellow) and Ko-Chi Cheng (Guest Researcher) came to LVD to receive postdoctoral training. Dr. Mark Challberg (Senior Staff Fellow) joined LVD to initiate a new program on herpesvirus DNA replication. Mary Rust was welcomed as an editorial assistant.

## Honors and Awards

Dr. Moss continues to serve on the editorial boards of the *J. of Virology*, *Virology*, and *J. of Biological Chemistry* and on the advisory board of *Advances in Virus Research*. He is a member of the advisory committee on *Nucleic Acid and Protein Synthesis* of the American Cancer Society and an advisor to the World Health Organization. During the past year he received an Inventor's Award from the U.S. Department of Commerce, delivered the Schultz Memorial Lecture at Stanford University and was elected to the board of directors of the Foundation for Advanced Education in the Sciences.

Dr. Levy was invited to serve as an editor of the Journal of Bioactive Polymers, as session chairman at interferon meetings in Heidelberg and Rome, and on steering group of the Decision Network Committee of the Biologic Response Modifier Program.

Members of LVD presented numerous invited lectures in this country and abroad.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00020-10 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on the Treatment of Disease with the Interferon System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H.B. Levy Section Head LVD, NIAID

COOPERATING UNITS (if any)
NCI, BRMP (M. Chirigos, J. Talmadge); N.Y.U. Cancer Ctr ( Muggia, Levin); U. Wisconsin Med. School (E. Border); Portsmouth Navel Hosp. (J. Reed); Childrens Cancer Testing Group (B. Lampkin); Walter Reed (A. Salazar); NINCDS (D. McFarlin).

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 1 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A meeting was held by the N.C.I. last year to which most of the people who have worked with Poly ICLC were invited. The one important conclusion from the clinical and preclinical observations is that Poly ICLC should be used at lower levels than we have been using it. The use of lower levels maximizes its immune enhancing activity; there being an optimum drug level above which there is actually decreased effectiveness and increased toxicity. It also was revealed that Poly ICLC is the most effective biologic response modifier that the N.C.I. has tested. Modest, but significant clinical improvement has been seen in juvenile laryngopapilloma, multiple myeloma and multiple sclerosis, but not leukemia, breast cancer or neuroblastoma at the high drug levels used.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00123-19 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Replication of Poxvirus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | M. Merchlinsky | Staff Fellow | LVD, NIAID |
| Others: | P. Earl | Senior Staff Fellow | LVD, NIAID |
| | B. Moss | Laboratory Chief | LVD, NIAID |

COOPERATING UNITS (if any)
LID, NIAID (Bahige M. Baroudy)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.4 | 1.6 | .8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Poxviruses provide a unique experimental system for studying DNA replication. The ends of the linear double-stranded DNA genome consist of hair-pin structures that may resemble telomeres of eukaryotic chromosomes. Enzymes and other proteins needed for DNA synthesis are encoded within the viral genome and replication occurs in the cytoplasmic compartment of infected cells. During the past year the DNA polymerase gene of vaccinia virus was completely sequenced and the primary structure of the enzyme was derived. A computer search of the National Biomedical Research Foundation protein data base revealed a significant homology with the DNA polymerases of Epstein-Barr virus and adenovirus. These data suggest that the DNA polymerases of poxviruses, herpesviruses and adenoviruses are related evolutionarily.

Previous studies from this laboratory demonstrated the presence of concatemeric forms of vaccinia virus DNA in infected cells. The junction region, between individual genome units in these concatemers was stably cloned in an Escherichia coli plasmid. Restriction endonuclease analysis indicated that the junction consists of an imperfect palindrome. The nucleotide sequence of the complementary strands was identical to that of the flip-flop hairpin loops at the ends of mature genomes. In cells that were infected with vaccinia virus, plasmids containing 250 bp or more of the junction were replicated and converted into minichromosomes with vaccinia DNA hairpins at each end and plasmid DNA in the center. An enzyme that carries out similar or identical cleavage and ligation reactions has been isolated and is being characterized.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00126-12 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | F.M. DeFilippes | Research Physicist | LVD, NIAID |
| Others: | G. Inchauspe | Visiting Fellow | LVD, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.35 | 1.25 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Vaccinia virus growth in BSC-1 or HeLa cells was inhibited by aphidicolin concentrations greater than 20 μM. After treatment of the wild-type virus with hydroxylamine, a viral mutant was isolated which is resistant to 80 μM aphidicolin. In an _in vitro_ assay viral DNA polymerase isolated from cells infected with mutant virus was more resistant to aphidicolin than viral DNA polymerase isolated from cells infected with wild-type virus. Transfer of aphidicolin-resistance was achieved by transfecting cells infected with wild-type virus with DNA isolated from aphidicolin-resistant virus. Production of aphidicolin-resistant recombinant viruses was measured by plaque assay in the presence of aphidicolin. The site of the mutation was initially located in the _Hind_III E segment of the resistant DNA. This segment, which was cloned in a pUC9 plasmid, was digested further with _Eco_RI. Marker rescue experiments with the resulting segments showed that the second largest, labeled _Eco_ B, contained the drug-resistance mutation. The _Eco_ B segment was cloned in pUC9 and the recombinant plasmid was digested with _Rsa_I. Transfection experiments with the entire digest showed a detectable level of aphidicolin-resistant plaques which was about ten times that found for a background produced by transfection with wild-type DNA. To determine which particular _Rsa_ segment contained the resistant site, the recombinant _Eco_ B plasmid was digested with exonuclease III, and the undigested DNA was tested for marker rescue. Correlation of the extent of exonuclease digestion with the map positions of the _Rsa_ sites indicated that a segment labeled _Rsa_ H might contain the mutation. This segment was cloned in pUC9 and the recombinant DNA was used to transfer the aphidicolin-resistance marker. Since the active _Rsa_ DNA segment is about 194 bp it should be easy to sequence with the M13 phage system.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00298-04 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Vaccinia Virus as an Expression Vector

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | B. Moss | Laboratory Chief | LVD, NIAID |
| Others: | K. Brechling | Staff Fellow | LVD, NIAID |
| | T. Fuerst | Staff Fellow | LVD, NIAID |
| | S. Chakrabarti | Visiting Associate | LVD, NIAID |
| | M. Buller | Visiting Associate | LVD, NIAID |
| | G. Kotwal | Visiting Fellow | LVD, NIIAD |

COOPERATING UNITS *(if any)*
CDC (J. Esposito); University of Washington (T. Yilma); LOM, NIDR (A.L. Notkins)

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Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.7 | 2.4 | 0.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Procedures have been developed for the use of vaccinia virus as a eukaryotic expression vector. A chimeric gene is formed by ligating vaccinia virus transcriptional regulatory signals to a foreign protein coding sequence. Homologous recombination is used to insert the chimeric gene into a non-essential region of the vaccinia virus genome. To facilitate the formation and isolation of recombinant virus, new plasmid vectors have been constructed which direct the insertion of the chimeric gene together with the E. coli β-galactosidase gene into the thymidine kinase locus. Recombinant virus is then selected on the basis of the thymidine kinase negative phenotype and/or staining with a β-galactosidase indicator dye. The recombinant viruses produced in this manner are stable and have a wide host range for tissue culture cells and animals. At least 25,000 base pairs of DNA can be inserted into the vaccinia virus genome without destroying infectivity. To optimize expression, the prokaryotic gene encoding chloramphenicol acetyltransferase was inserted into vaccinia virus under control of different promoters. This system has been used to express genes from a variety of infectious agents including herpes simplex virus type 1, hepatitis B virus, influenza virus, vesicular stomatitis virus and rabies virus. Animals vaccinated with each of the above recombinants were protected against challenge with the corresponding virus.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00306-04 LVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Pathogenesis of Orthopoxvirus Infections

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | R.M.L. Buller | Visiting Associate | LVD, NIAID |
| Others: | B. Moss | Laboratory Chief | LVD, NIAID |
| | G. Kotwal | Visiting Associate | LVD, NIAID |

COOPERATING UNITS (*if any*)
LIP, NIAID (H.C. Morse,III); ATCC (  Weinblatt); LCBGY (M. Potter); University of Conn. (T. Fredrickson); Montreal General Hospital, Canada (E. Skamene).

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Macromolecular Biology Section

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| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | 1 | 0.25 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Ectromelia, an orthopoxvirus, causes mousepox in colonized mice. Studies were carried out: 1) to improve the surveillance and control measures necessary to prevent future mousepox epizootics in the mouse colonies; and 2) to study the molecular basis of orthopoxvirus pathogenesis in inbred mice, and to apply the acquired knowledge towards the development of a safe, effective recombinant vaccinia virus vaccine for human use.

1) Mousepox control measures: These studies showed that the current practice of immunizing mice against mousepox with vaccinia virus IHD-T is of dubious value and should only be carried out in exceptional circumstances. It was also demonstrated that all cell lines (especially hybridomas) passed in mice potentially can become infected with ectromelia virus, and can result in new cases of mousepox on reinoculation into previously unexposed mice.

2) Virus pathogenesis studies: This research was expanded this year and carried out in two mutually supportive directions, firstly a number of different regions of the orthopoxvirus genome were evaluated for their contribution to virus virulence in mice and rabbits. The thymidine kinase, and vaccinia growth factor genes were shown to contribute to virus virulence in the host, as well as a 9 kb protein coding region proximal to the left hand terminus of the genome. The second area of investigation involved the genetic analysis of inbred mouse strains in order to determine the number and importance of non-H-2 genes responsible for recovery from mousepox. The resistance genes segregated as a multifactorial dominant locus, and a portion of the resistance phenotype appeared to be sex-linked. Subsequent work will focus on the mechanism by which these genes protect the host from mousepox.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00307-04 LVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | B. Moss | Laboratory Chief | LVD, NIAID |
| Others: | J.P. Weir | Senior Staff Fellow | LVD, NIAID |
| | S. Chakrabarti | Visiting Associate | LVD, NIAID |
| | S. Broyles | Staff Fellow | LVD, NIAID |
| | J. Rosel | Visiting Fellow | LVD, NIAID |
| | L. Yuen | Visiting Fellow | LVD, NIAID |
| | G. Rohrmann | Expert | LVD, NIAID |
| | N. Elango | Visiting Associate | LVD, NIAID |

COOPERATING UNITS (*if any*)

Smith, Klein and French Laboratories (E.V. Jones)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

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NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.0 | 5.5 | .5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Vaccinia virus has a genome of 185,000 bp that encodes approximately 200 polypeptides. These genes are expressed in a coordinated fashion so that some polypeptides are made before and others after DNA replication. One vaccinia virus gene that is expressed throughout the growth cycle was found to have two RNA start sites about 55 bp apart. The site nearest to the coding segment was used at early times in infection and the other was used at late times. In vitro mutagenesis studies revealed that there are two independent promoters and that the regulatory signals are located within 31 bp of each RNA start site.

RNA polymerase subunits, synthesized in reticulocyte lysates programmed with early vaccinia virus mRNA, were immunoprecipitated by antibody prepared against the purified enzyme. The subunit genes were mapped by hybridization selection of mRNA to cloned DNA fragments prior to translation. The genes for 2 subunits of 147 kD and 22 kD were sequenced. The large subunit was shown to have considerable homology with the β' subunit of *E. coli* RNA polymerase.

A soluble extract capable of selectively transcribing added early vaccinia virus genes was prepared by disrupting purified vaccinia virus particles. Correct initiation, termination and polyadenylation were demonstrated and the signals for each were defined by transcription of truncated templates. Termination was shown to occur about 50 bp downstream of the signal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00391-02 LVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Expression of Genes of Respiratory Viruses by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | N. Elango | Visiting Associate | LVD, NIAID |
| Others: | B. Moss | Laboratory Chief | LVD, NIAID |
| | G.L. Smith | Visiting Associate | LVD, NIAID |

COOPERATING UNITS (*if any*)

The Wistar Institute (J.R. Bennink and J.W. Yewdell); LID, NIAID (B. Murphy, P. Collins, and R. Chanock); University of Florida (P.A. Small)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | .7 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Development of effective vaccines against respiratory viruses requires a thorough understanding of the immunological response to individual proteins. Recombinant vaccinia viruses have been constructed that express individual influenza virus genes. Tissue culture cells infected by vaccinia recombinants expressing haemagglutinin (HA-VAC) or nucleoprotein (NP-VAC) synthesized authentic influenza polypeptides which were expressed on the cell surface and which were recognized by anti-influenza A virus cytotoxic T lymphocytes (CTL) in a H-2 restricted manner. Animals vaccinated with HA-VAC recombinants produced antibodies against HA (anti-HA) and were protected against subsequent intranasal challenge with influenza virus of the homologous subtype. Mice immunized with HA-VAC or NP-VAC were primed for secondary anti-HA or anti-NP CTL responses, respectively. Anti-HA CTL were mostly subtype specific while anti-NP CTL were strongly crossreactive among all influenza A virus subtypes.

A cDNA copy of the glycoprotein G gene of respiratory syncytial virus has been inserted into the vaccinia virus genome. A glycosylated protein of 84 kD was synthesized and immunoprecipitated with specific antiserum. Transport to the cell surface was demonstrated by immunofluorescence.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00392-02 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Expression of Hepatitis B Virus Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. Brechling | Staff Fellow | LVD, NIAID |
| Others: | B. Moss | Laboratory Chief | LVD, NIAID |
| | G.L. Smith | Visiting Associate | LVD, NIAID |

COOPERATING UNITS *(if any)*
New York University (K.C. Cheng); LID, NIAID (R. Purcell); Georgetown University (J. Gerin)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.3 | 1.0 | 0.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


Hepatitis is a serious world-wide health problem. Approximately 200 million people are chronically infected with hepatitis B virus and large numbers of deaths are attributed to fulminant hepatitis, cirrhosis, and hepatocellular carcinoma. Although an effective subunit vaccine has been produced, limitations in supply and expense have prevented its global use. As an alternative, we are trying to construct a live recombinant hepatitis B vaccine. The gene for hepatitis B virus surface antigen has been engineered and inserted into the genome of vaccinia virus. The recombinant vaccinia virus is stable and expresses high levels of the hepatitis virus protein. The latter is glycosylated, assembled into particles and transported through the plasma membrane of infected cells. Rabbits, vaccinated with the recombinant virus, produce a high and sustained specific antibody response. Vaccination of chimpanzees resulted in priming of the immune system and protection against clinical hepatitis upon subsequent challenge with hepatitis B virus. Vaccinia virus recombinants that express higher levels of HBsAg have been constructed by using the promoter from a major structural protein of vaccinia virus and are being evaluated.

Recent studies have indicated that the DNA sequence preceding the HBsAg gene, referred to as pre-S, is expressed by hepatitis B virus and contains immunologically dominant epitopes. A vaccinia virus recombinant that expresses the entire long open-reading-frame was constructed. Rabbits vaccinated with this recombinant produced antibodies to pre-S and S epitopes

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00393-02 LVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Expression of Malaria Genes by Vaccinia Virus Recombinants

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | B. Moss | Laboratory Chief | LVD, NIAID |
| Others: | G.L. Smith | Visiting Associate | LVD, NIAID |

COOPERATING UNITS (*if any*)

New York University (K.C. Cheng, R. and V. Nussenzweig); Walter and Eliza Hall Institute (C. Langford); LPD, NIAID (T. McCutcheon and L. Miller)

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Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .55 | .25 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)


Malaria remains a serious global health problem for which there is no effective vaccine. Previous studies indicate that animals can be immunized with inactivated sporozoites. The genes coding for the circumsporozoite antigens of the malaria parasite Plasmodium knowlesi and P. falciparum were inserted into the vaccinia virus genome under the control of a defined vaccinia virus promoter. Tissue culture cells infected with the recombinant synthesized polypeptides that reacted with monoclonal antibody against the malaria protein. Studies on the sequence of the expressed P. falciparum CSP indicated that the $NH_2$-terminus is blocked and COOH-terminus is not processed. Immunofluorescent staining demonstrated that the CSP was distributed primarily in the cytoplasm of infected cells. Rabbits vaccinated with the recombinant virus produced antibodies that bound specifically to sporozoites.

The S antigen gene of P. falciparum also was expressed in a vaccinia virus recombinant. The protein was secreted from infected cells and reacted with specific antiserum. Vaccinated animals produced a low but detectable antibody response.




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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00416-02 LVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Recombinant Vaccines Against Retroviruses Associated with Leukemia and AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | S. Chakrabarti | Visiting Associate | LVD, NIAID |
| Others: | P. Earl | Senior Staff Fellow | LVD, NIAID |
| | B. Moss | Laboratory Chief | LVD, NIAID |

COOPERATING UNITS (if any)

LPVD, NIAID (B. Chesebro); LTCB, NCI (F. Wong-Staal and R. Gallo)

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Laboratory of Viral Diseases

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.2 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Retroviruses, long associated with leukemia and sarcoma of animals, have recently been implicated as the etiological agents of human T cell leukemia and human acquired immune deficiency syndrome (AIDS). The identification of these agents makes it possible to consider various ways of prevention. The most promising approach is development of a vaccine that could be administered to individuals at risk. The vaccinia vector system has been shown to produce both humoral and cell mediated immunity against a variety of infectious agents. Since human retroviruses have not yet been shown to produce disease in animals, initial vaccine work must be done with animal retroviruses. Friend leukemia virus complex is a useful model system since it produces an acute disease in adult mice which can be prevented by repeated immunization with the envelope glycoprotein. The envelope gene of Friend murine leukemia virus (MuLV) was inserted into vaccinia virus under the control of a vaccinia virus promoter that is active at early and late times after infection. Pulse-labeling experiments indicated that an MuLV polypeptide of 85 kD was synthesized and subsequently processed to polypeptides of 70 kD and 15 kD. Immunofluorescence studies indicated that the 70 kD polypeptide was inserted into the cell membrane. Mice vaccinated with the recombinant virus produced antibodies to the MuLV envelope protein and were protected against the development of splenomegaly upon intravenous challenge with MuLV

A similar procedure was used to prepare vaccinia virus recombinants that express the envelope gene of HTLV-III, the causative agent of AIDS. Immunoblotting and immunoprecipitation studies indicated that the 160 kD polypeptide is synthesized and processed correctly. The ability of this recombinants to induce an immune response in experimental animals is under investigation.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00443-01 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vaccinia Virus Growth Factor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | S. Chakrabarti | Visiting Associate | LVD, NIAID |
| Others: | B. Moss | Laboratory Chief | LVD, NIAID |

COOPERATING UNITS *(if any)*
ONCOGEN (D. Twardzik); Fred Hutchinson Research Laboratories (J. Cooper); Microgenysis (M. Cochran);

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.4 | 0.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The recent discovery, that a vaccinia virus (VV) gene encodes a polypeptide with structural homology to transforming growth factor (TGF-α) and epidermal growth factor (EGF), led us to look for a virus-induced protein with the predicted biological activity. The supernatant of VV infected cells cultures were found to contain an acid stable $M_r$ 25,000 polypeptide which competes with EGF for binding to EGF membrane receptors. This vaccinia virus-induced growth factor (VGF) like EGF and TGF-α is mitogenic and stimulates anchorage independent cell growth in the presence of TGF-β. However, VGF did not cross-react in a radioimmunoassay specific for small and large forms of TGF-α and exhibited minimal cross-reactivity with antisera to EGF. VGF was detectable in the culture medium within 2 hr and maximal amounts were present 12 hr after infection. The level of VGF was proportional to the multiplicity of VV used. Inhibition of viral DNA synthesis enhanced VGF production, consistent with the hypothesis that VGF is an early gene product encoded by VV. The demonstration of a novel growth factor, that is released from cells infected with vaccinia virus, may have important implications regarding virus-host interactions. In order to determine the nature of these interactions, a VV mutant that does not produce VGF was isolated and is being studied.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00444-01 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Expression of Herpes Simplex Virus genes.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.P. Weir Senior Staff Fellow LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.3 | 0.2 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The expression of the HSV1 glycoprotein C gene was studied by cloning the promoter for this gene next to the coding sequences for the bacterial enzyme, β-galactosidase. The expression of β-galactosidase activity was directed by the glycoprotein C gene promoter and the level of β-galactosidase activity was used as a measure of the promoter activity. Mammalian cell lines were transfected with this plasmid construction or with an identical plasmid construction minus the promoter sequences and assayed for galactosidase activity at 48 hours. The plasmid without a promoter never showed galactosidase activity. The plasmid with the glycoprotein C promoter expressed galactosidase activity but only when the cells were also infected with HSV1. β-galactosidase was also made from this plasmid if, instead of being infected with HSV1, the cells were co-transfected with a plasmid recombinant containing two of the HSV1 immediate-early genes. This transient assay system will be useful for determining what sequences in the glycoprotein C promoter are important in regulation.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00445-01 LVD

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Viral DNA Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M. Challberg Senior Staff Fellow LVD, NIAID

COOPERATING UNITS *(if any)*
Tufts University Medical School (J. Bernstein); Johns Hopkins School of Medicine (T. Kelly)

LAB/BRANCH
Laboratory of Viral Diseases

SECTION
Macromolecular Biology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.670 | 0.5 | 0.17 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human adenoviruses and herpesviruses are being used as model systems for studying DNA replication in eukaryotic cells. In the past year, we have continued with a genetic analysis of the initiation of adenovirus DNA replication. We have previously shown that the origin of adenovirus DNA replication is comprised of two functionally distinct domains: a ten base pair sequence which probably represents the binding site for a viral initiation protein and an adjacent 20 base pairs which constitutes the binding site for a cellular protein, Nuclear Factor I. We have concentrated in particular on the Nuclear Factor I binding site. Using oligonucleotide mutagenesis we have constructed plasmids with point mutations in the binding site region. Studies with these mutants both _in vitro_ and _in vivo_ have established the following conclusions: 1) the nucleotide sequence specifically recognized by Nuclear Factor I is TGG(N6)GCCAA; 2) the Nuclear Factor I binding site is required for replication _in vivo_ as well as _in vitro_ 3) tight binding of Nuclear Factor I to the origin is a necessary, but not sufficient condition for initiation _in vitro_.

We have just begun work with HSV-1. Our efforts to date have concentrated on an attempt to develop a useful _in vitro_ system for studying HSV-1 DNA replication.



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LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
Rocky Mountain Laboratories
Hamilton, Montana
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Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984, to September 30, 1985

RESEARCH HIGHLIGHTS

Major emphasis in LMSF is definition of structural and functional elements of pathogenic bacterial surface components involved in pathogenesis and/or virulence of selected organisms or in genesis of hosts' immunological responses to infections by these agents. Primary attention is directed toward both extracellular pathogens (including Neisseria gonorrhoeae, Contagious Equine Metritis Organism, and Borrelia burgdorferi) and intracellular parasites (including Chlamydia trachomatis, Chlamydia psittaci, Rickettsia rickettsii, and Coxiella burnetii). Both protein and nonprotein components of these gram-negative organisms' outer membrane are prime study candidates as mediators of interactions between bacterium and host and as likely vaccine components. Chemical characteristics, immunochemical properties, and genetic control of selected surface components are investigated to delineate their relationship to infectious disease phenomena of these bacteria. A number of findings emanating from studies in LMSF during the past year are summarized below:

Chlamydiae: The major outer membrane protein (MOMP) of chlamydiae has been found to be a major immunogen against which antibody responses of the host are directed during natural or experimental infection. Monoclonal antibodies have been developed by Caldwell to detect species-specific, subspecies-specific, and type-specific epitopes of MOMP; that work has been expanded by Zhang in Caldwell's lab with regard to serotypes of chlamydiae responsible for endemic trachoma in China (Peoples' Republic). Immunoelectron microscopy has demonstrated that species- and subspecies-specific epitopes are relatively inaccessible to antibody on the chlamydial surface whereas type-specific epitopes are highly exposed. Only monoclonal antibodies directed against the type-specific epitope of MOMP are capable of interfering with chlamydial infection of tissue culture cells, in vitro; this type-specific epitope has been localized to one cyanogen bromide fragment (15 kd) of MOMP. Molecular cloning of the MOMP type-specific epitope encoding-DNA from C. trachomatic serotype L2 and from C. psittaci GPIC serotype is in progress by Nano and Caldwell. DNA coding for each of the major surface proteins of GPIC (see below) has also been cloned into E. coli by Nano and Caldwell; entire plasmids from both C. trachomatis and C. psittaci have been cloned by Joseph and Nano to compare their comparative structures and products. Also being cloned by Nano is DNA which encodes proteins that bind HeLa cell membrane components, as identified recently by Hackstadt; these surface components appear on infectious chlamydial elementary bodies but are absent from metabolically-active, noninfectious chlamydial reticulate bodies. These HeLa cell surface-binding chlamydial components appear to bind heparin, and their HeLa component binding activity is sensitive to reducing agents or protease inhibitors; they are the likely outer membrane constituents responsible for chlamydial binding to host cell plasmalemmas and for desorption or detachment of chlamydiae from host cells by heparin. Nano previously cloned DNA that encodes an epitope of chlamydial LPS which is common among different species and serotypes of chlamydiae. This cloned DNA has potential diagnostic use and is also being considered as a vaccine candidate on the basis of preliminary work (in collaboration with Taylor, Johns Hopkins Univ.) showing some "protection" of subhuman primates against clinical manifestations of trachoma upon infection with chlamydiae; relevant to this latter phenomenon, the LPS epitope-encoding DNA has been transferred into

various LPS chemotypes of Salmonella typhimurium including an aro deletion mutant and also into vaccinia virus. One of the most exciting pieces of work done in LMSF during the past year utilizes a guinea pig host and a strain of C. psittaci (GPIC) as the infectious agent that causes guinea pig inclusion conjunctivitis. Watkins and Caldwell have examined this model system in several ways. First, they found that initial conjunctivitis due to GPIC spontaneously "cleared" in about 2 weeks and rendered the guinea pig refractory to subsequent challenge infection by relatively small numbers of the same organism; however, larger numbers of GPIC chlamydia did elicit an inflammatory conjunctivitis. A Triton X-100 extract of GPIC chlamydiae could replicate this inflammatory conjunctival reaction in previously-infected guinea pigs and the response resembles classical trachoma in several ways. The chlamydial component that evokes both this conjunctival response and a cutaneous hypersensitivity reaction is being identified on intact chlamydiae, in detergent extracts, and in E. coli bearing recombinant plasmids (with GPIC DNA). Preliminary data indicate that a 50-kd protein may be the chlamydial moiety responsible for conjunctival and cutaneous hypersensitivity reactions; it is clearly not chlamydial LPS. Histopathological changes accompanying both primary chlamydial conjunctival infection and the subsequent hypersensitivity reactions are being investigated by Hadlow (LPB) in collaboration with Watkins and Caldwell. Additional work done by Caldwell and Watkins in collaboration with Taylor (Johns Hopkins) suggests that similar Triton extracts from chlamydiae of relevant serotype will provoke analogous reactions in nonhuman primate eyes following initial sensitization with an active chlamydial infection. These findings suggest that the primary guinea pig conjunctivitis and subsequent hypersensitivity system will be useful for understanding the pathobiology of trachoma. Work is underway by Joseph and Caldwell to characterize the surface components of GPIC chlamydiae in detail similar to that done previously with C. trachomatis. Having devised a satisfactory method for chemical extraction and purification of LPS from chlamydiae, Hitchcock has undertaken a detailed analysis of this outer membrane component by biochemical and biological methods. Chlamydial LPS is nontoxic for chick embryos, contains KDO and lipid A, and exhibits unusual fatty acids as well as glucose and glucosamine. The epitope recognized by monoclonal antibodies appear non-exposed on the organism's exterior.

Spotted fever rickettsiae: Attempts to identify components of Rickettsia rickettsii that stimulate protective immunity are being continued and have produced encouraging results during the past year. Anacker has developed an extensive bank of monoclonal antibodies in mice infected with viable R. rickettsii; these show three different specificities, recognizing epitopes located on 1) LPS, 2) a 120-kd protein, and 3) a 155-kd protein. Anti-LPS monoclonal antibodies exhibit no protective activity when administered to mice prior to challenge with two $LD_{50}$'s of viable rickettsiae. Anti-155-kd protein monoclonal antibodies protected mice from lethal challenge, but all strains of spotted fever rickettsiae do not have the same 155-kd protein as shown by immunoblotting, and protective activity of monoclonals is manifest only on challenge with rickettsiae bearing the 155-kd proteins that react with a particular monoclonal. The monoclonal reactive with the 120-kd protein protects mice from lethal challenge. Also, rickettsial extracts enriched for this protein lead to (partial) active immunologic protection against similar challenge in mice. Spotted fever rickettsiae of differing virulences have also been identified, and their surface components are being analyzed to provide additional reagents for understanding virulence of these organisms. DNA prepared from rickettsiae has been used for molecular cloning by McDonald who demonstrated that one clone, pGM19, constituted

a partially protective vaccine when E. coli bearing the recombinant clone were administered to mice prior to lethal challenge; this recombinant produces a 28-kd protein that is recognized by immunoprecipitation with a monoclonal (from Anacker's collection) which recognizes a 120-kd protein on intact rickettsiae.

Coxiella burnetii (Q-fever agent): During the past year, Hackstadt has clearly demonstrated that phase I (virulent) versus phase II (avirulent) C. burnetii differ in their LPSs with a "smooth" LPS form found for the former and a "rough" LPS seen in the latter. Further, he has defined an intermediate LPS in a C. burnetii isolated from chronic ovine infection; this was followed by examination of C. burnetii strains derived from chronic human infections (endocarditis and hepatitis). These chronic infection isolates have immunochemically distinct LPS species based on studies with these organisms and sera from individuals harboring these agents. Comparative virulences of these C. burnetii with different LPSs are being studied in animal models to further understand the role of LPS (which is nontoxic for chick embryos) in pathogenicity/virulence of these intracellular parasites.

Lyme disease spirochete (Borrelia burgdorferi): Howe has continued to analyze molecularly-cloned B. burgdorferi DNA which encodes two major outer membrane proteins and directs synthesis of these proteins in E. coli. This clone has been examined by transposon mutagenesis and subcloning to localize the ospA and ospB structural genes and to define whether their polypeptide products are transcribed coordinately. In addition, several strains of the Lyme disease spirochetes of domestic and foreign origin have been compared by Southern blot-hybridization methods; the results have defined polymorphism for ospA encoding DNA among this battery of isolates.

Contagious Equine Metritis Organism (CEMO): During the past year, Hitchcock has continued her attempts to separate, purify, and characterize both LPS and capsular elements of this equine pathogen. Identification of this organism's capsule has used scanning and transmission electron microscopy since large aggregates of capsular substance can be visualized with the fixation and examination techniques which have been developed. The immunochemical characterization of LPS is being pursued.

Gonococci: Both protein and nonprotein components of the gonococcal (Gc) outer membrane are being studied in LMSF. The "elusive" Gc capsule, previously detected only by light microscopy, is being sought by Hitchcock through use of immunologic techniques, isolation and chemical characterization, and electron microscopic methods. A high molecular weight polymer has been isolated, contains mainly (85%) neutral sugar, and is antigenic (with hyperimmune rabbit serum). Electron microscopy reveals amorphous material which, by analogy with CEMO capsule's appearance, may be capsule. Hitchcock and Strittmatter are characterizing an antigen--H8--that appears to occur among all Gc as well as meningococci, but not commensal neisseriae. This H8 antigen varies in subunit size among different strains but retains a common epitope detectable by a monoclonal antibody. Initial biochemical analysis reveals two fatty acid-like constituents and a peptide component devoid of aromatic amino acids and methionine. This antigen is being evaluated for its possible role both as an adherence-mediating component and in relation to resistance of killing by immune sera. The heterogeneity of LPS species among different strains and intrastrain variants of Gc is being explored by Hitchcock through use of electrophoretic and immunochemical methods as well as by chemical characterization. Several distinct forms of Gc LPS have been identified. Also being continued by Hitchcock are studies on interactions between LPS and outer membrane porin protein I of Gc.

Studies on genetic control of piliation in Gc are being pursued by Swanson and Bergstrom. $Pilus^-$ Gc were identified differentiated on their abilities to revert or not (to $pilus^+$); this correlated with deletion-causing rearrangements of pilin gene DNA for nonreverting phenotypes whereas reverting $pilus^-$ Gc did not show such rearrangements. Sequence analysis of pilus subunit-specifying mRNA of reverting $pilus^-$ Gc revealed that some had accumulated "ochre" mutations (premature termination signals) and produced truncated pilin subunits; other reverting $pilus^-$ Gc produced full-length pilin which had distorted secondary structures (probably precluding their assembly into mature pili) due to insertion of codons for prolyl residues. Both of these "abnormal" pilin gene sequences underwent recombination with appearance of "orthodox" pilin gene sequences on reversion to $pilus^+$ phenotype. Also investigated were Gc bearing two copies of the pilin structural gene; these organisms displayed a myriad of rearrangements involving their pilin gene DNA, but these events did not lead to loss of $pilus^+$ phenotype. These observations provide evidence that at least three different events that effect loss of $pilus^+$ phenotype may eventuate during intragenomic recombination between complete pilin gene and the multitude of partial pilin gene copies in the Gc chromosome. Additional studies are being carried out with $rec^-$ Gc constructed by Koomey, and preliminary evidence points to additional, recombination-independent mechanisms through which Gc change either their piliation status and/or the antigenic forms of these Gc cell wall appendages.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

ADMINISTRATIVE REPORT

Personnel changes during the past year include transfer of Dr. Alan Barbour to LPB, NIAID, and departure of Dr. Leonard Mayer (to CDC, Atlanta) and Dr. Paul Barstad (to Gull Labs, Salt Lake City). Two members of LMSF technical staff departed (S. Tessier and M. Schrumpf) and one new technician was added (L. Milch). In the absence of a NIAID Summer Student program, only two undergraduate students worked in LMSF this year--Ms. Kareen Garjian (University of California, Berkeley) and Ms. Patricia Sullivan (Carroll College, Helena, MT). Seminars were given by visitors as follows: Dr. A. Bahrmand (Copenhagen); Dr. P. Hagblom (Scripps, San Diego); Dr. J. Hazelbauer (Washington State University, Pullman); Dr. J. James (University of Hawaii, Honolulu); Dr. M. Koomey (Rockefeller University, New York); Dr. L. Leive (NIADDK, Bethesda); Mr. B. Lund (University of Umea, Sweden); Drs. Fritz and Ida Orskov (Statens Seruminstitut, Copenhagen); Dr. L. Randall (Washington State University, Pullman); Dr. C. Schnaitman (University of Virginia, Charlottesville); Dr. S. Normark (University of Umea, Sweden); Dr. G. Dasch (NMRI, Bethesda); and Dr. H. Brade (Borstel, FRG).

Annual Report
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Hamilton, Montana
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October 1, 1984, to September 30, 1985

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Infection and Immunity
P. Hitchcock - Journal Bacteriology

Manuscripts were reviewed by LMSF staff for the following journals: Canadian Journal of Microbiology, Infection and Immunity, Journal of Bacteriology, Journal of Clinical Investigation, Journal of Clinical Microbiology, Journal of General Microbiology, Journal of Immunology, Journal of Infectious Diseases, Proceedings of the National Academy of Sciences, USA, Science, and Sexually Transmitted Diseases.

Professional Posts:

J. Swanson - Member, Microbiology and Infectious Diseases Research Committee, NIAID
Member, NIAID Promotions & Tenure Committee

H. Caldwell - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

T. Hackstadt - Consultant Member, Food and Drug Administration, Orphan Products Development Initial Revew Group, Rockville, MD

Invited Lectures and Participation in Meetings and Symposia:

J. Swanson - Gordon Conference on Bacterial Cell Surfaces, Plymouth, NH
Conference on Pathogenic Neisseriae, Asilomar, CA
ASM Northwest Branch Annual Meeting, Pullman, WA

H. Caldwell - First Darwin Conference on Trachoma and Chlamydial Disease, Darwin, Australia

T. Hackstadt - Stanford University, Stanford, CA

P. Hitchcock - Gordon Conference on Bacterial Cell Surfaces, Plymouth, NH
University of Kansas, School of Medicine, Kansas City, KS
Washington State University, Pullman, WA
List Biological Labs, Inc., Campbell, CA
Abbott Labs, Chicago, IL

T. Howe - Gordon Conference on Bacterial Cell Surfaces, Plymouth, NH
Oregon Health Sciences University, Portland, OR

F. Nano - Cetus Corporation, Emeryville, CA
University of Montana, Missoula, MT
University of Washington, Seattle, WA

Other Activities:

J. Swanson - Reviewed research grants for National Science Foundation, Washington, DC, and Medical Research Council of Canada, Ottawa, Canada

H. Caldwell - Reviewed research grants for National Science Foundation, Washington, DC, British Columbia Health Care Research Foundation, British Columbia, Canada, and The Edna McConnell Clark Foundation, New York, NY

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00065-12 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigens and Classification of Rickettsiae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. L. Anacker | Research Microbiologist | LMSF, NIAID |
| Others: | G. A. McDonald | Staff Fellow | LMSF, NIAID |
| | W. Burgdorfer | Research Entomologist (Med.) | LPB, NIAID |

COOPERATING UNITS (if any)

Dr. K. E. Hechemy, New York State Department of Health, Albany, NY

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.0 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Efforts have been continued to identify and characterize those constituents responsible for the biological activities of the etiologic agent of Rocky Mountain spotted fever, Rickettsia rickettsii. Studies with various kinds of extracts suggested that the 120 K surface protein was the principal protective antigen. The 120 K protein-rich fractions from affinity columns stimulated in mice much better active immunity than did the original extract. The 120 K protein was heat labile; it was detected in immunoblots with monoclonal antibodies after exposure to temperatures of 25°C but not 37°C. Intact rickettsiae held at 56°C for 15 min did not induce protective immunity in mice. It was shown by crossed radioimmuno-electrophoresis and immunoblotting with monoclonal antibodies that R. rickettsii LPS, erythrocyte-sensitizing substance (ESS), and the complement fixing (CF) antigen were all derivatives of the same rickettsial antigen. Though the 120 and 155 K proteins of R. rickettsii strains of high and low virulence for guinea pigs had identical relative mobilities and common epitopes, these proteins were structurally different. The same number of rickettsiae of high and low virulence strains was required to kill mice in less than 24 h, so it does not appear that the ability of rickettsiae to kill mice in this type of assay is a major factor in rickettsial virulence for guinea pigs and man. Guinea pigs inoculated with a low virulence strain, the Iowa strain, exhibited few or no symptoms of disease but were subsequently immune to challenge with a high dose of the virulent R strain. SDS-PAGE analysis revealed that the Iowa strain lacked a 32 K heat-modifiable protein found in other strains and had a ~140 K protein instead of the 120 K protein described above. It is not presently known whether these changes in protein composition are related to the loss of virulence of the Iowa strain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00193-06 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J. Swanson | Chief | LMSF, NIAID |
| Other: | S. Bergstrom | Visiting Fellow | LMSF, NIAID |
| | O. Barrera | Microbiologist | LMSF, NIAID |
| | K. Robbins | Microbiologist | LMSF, NIAID |

COOPERATING UNITS *(if any)*

Michael Koomey, Rockefeller University, New York

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 2.25 | 2.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Surface properties of gonococci (Gc) are markedly influenced by their possessing pili whose structural/functional characteristics may vary widely. Gc exhibit high-frequency changes both in their piliation status (pilus$^+$ or pilus$^-$) and in the pilin subunits which constitute their mature pili. Both "on-off" transitions and subunit size variation of Gc pili are being studied to discover their control mechanisms. These transitions were examined for colonial morphotype, piliation (by SEM), pilin production and subunit size (by immunoblotting), pilin-mRNA (by Northern blotting), pilin gene-containing DNA rearrangements (by Southern blotting), cloning and sequencing of Gc pilin gene DNA, and mRNA sequencing. We have identified and differentiated several pilus$^-$ phenotypes--both reverting (to pilus$^+$) and nonreverting--and have identified the changes in pilin gene sequence that accompany their lack of ability to produce pilin subunit that can be incorporated into bonafide pili of Gc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00194-05 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Neisseria gonorrhoeae

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. W. Mayer Expert (Microbiology) LMSF, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

L. W. Mayer transferred to Centers for Disease Control, Atlanta, Georgia, in September 1985, therefore this project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00216-05 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunochemistry of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. D. Caldwell | Research Microbiologist | LMSF, NIAID |
| Others: | F. E. Nano | Staff Fellow | LMSF, NIAID |
| | N. G. Watkins | Staff Fellow | LMSF, NIAID |
| | Y.-X. Zhang | Visiting Fellow | LMSF, NIAID |
| | T. Joseph | Graduate Student | LMSF, NIAID |

COOPERATING UNITS (*if any*)

Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.8 | 1.4 | 1.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Surface antigens that confer serotype specificity to Chlamydia trachomatis isolates are believed to function as protective antigens. The objective of this project is to identify the surface component(s) that possess these serotyping determiners with the rationale that they would be primary candidate antigen(s) for a subunit or live recombinant chlamydial vaccine. Monospecific polyclonal antisera and monoclonal antibodies have been raised against the chlamydial major outer membrane protein (MOMP) and characterized with respect to their specificity and function. The findings show that MOMP is the primary serotyping antigen of C. trachomatis possessing antigenic determinants of type, subspecies, and species specificity. These serologic properties were corroborated both by one- and two-dimensional peptide mapping of V8 protease and αchymotrypsin-digested MOMP. Immunoelectron microscopy studies with MOMP monoclonal antibodies showed the MOMP type-specific epitope to be highly exposed on the chlamydial cell surface. Type-specific MOMP antibodies were capable of neutralizing in vitro infectivity. The type-specific epitope has been located on a 15Kd cyanogen bromide MOMP peptide fragment. Collaborative studies are being focused on the molecular cloning of the MOMP gene and developing chlamydial animal models in order to directly ascertain the role of the MOMP as a protective antigen.



PHS 6040 Rev. 1/84 GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00230-03 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Virulence-Associated Factors of Rickettsia rickettsii

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: R. L. Anacker Research Microbiologist LMSF, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project has been combined with Project No. Z01 AI 00065-12 LMSF.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00231-04 LMSF |
|---|---|

PERIOD COVERED
October 1, 1984, to February 10, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Relapsing Fever Borrelia spp.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. G. Barbour | Senior Staff Fellow | LMSF, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
A. G. Barbour transferred to Laboratory of Pathobiology in February 1985, therefore this project has been transferred to LPB.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00232-04 LMSF

PERIOD COVERED

October 1, 1984, to February 10, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Pathogenesis of Lyme Disease

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. G. Barbour Senior Staff Fellow LMSF, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

A. G. Barbour transferred to Laboratory of Pathobiology in February 1985, therefore this project has been transferred to LPB.



PHS 6040 Rev. 1/84

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00233-03 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Structural Analysis of the Major Outer Membrane Protein of *Chlamydia trachomatis*

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: H. D. Caldwell Research Microbiologist LMSF, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project has been combined with Project No. Z01 AI 00216-05 LMSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00234-04 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Biology of Intracellular Parasitism

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | T. Hackstadt | Senior Staff Fellow | LMSF, NIAID |
| Others: | A. B. Moos | Staff Fellow | LMSF, NIAID |
| | H. D. Caldwell | Research Microbiologist | LMSF, NIAID |

COOPERATING UNITS (*if any*)

David Paretsky, University of Kansas
Mark Peppler, University of Alberta

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.75 | 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project examines mechanisms of pathogenesis of obligately intracellular bacteria. Initial events of Chlamydia-host interaction, including attachment, internalization, inhibition of phagosome-lysosome fusion, and differentiation are being examined. Study of the attachment and penetration phases of chlamydia with eucaryotic cells required an efficient means of distinguishing bound from internalized parasites. We assayed agents that had been used to release viruses or polypeptide hormones from the cell surface and found that heparin most effectively released bound chlamydia. The technique of heparin release was coupled with temperature shift experiments to examine a number of treatments of chlamydia or host on the uptake of chlamydial elementary bodies. We have identified two chlamydial proteins, present on the infectious stage of the life cycle but absent from the noninfectious form, that bind host cell surface protein. These two proteins also bind heparin, are sensitive to reducing agents and/or protease inhibitors and vary between Chlamydia trachomatis serotypes in correlation with disease caused. These proteins possess a number of properties that, collectively, are suggestive of a role in host-parasite interaction. A different approach has been taken in the study of Coxiella burnetii. This rickettsia undergoes a serologically defined virulent to avirulent phase transition. Our studies have demonstrated that the component that is structurally and antigenically unique between phases is the lipopolysaccharide. Consideration of the serological definition of phase in the context of LPS variation allowed us to identify a previously unknown LPS chemotype intermediate in structural complexity to the virulent and avirulent LPS types. This intermediate type LPS should prove useful in understanding the structure and function of C. burnetii LPS.



PHS 6040 (Rev. 1/84)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00235-04 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Nonprotein Surface Constituents of Three Venereal Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | P. J. Hitchcock | Senior Staff Fellow | LMSF, NIAID |
| Others: | W. G. Strittmatter | Visiting Fellow | LMSF, NIAID |
| | H. D. Caldwell | Research Microbiologist | LMSF, NIAID |
| | T. Hackstadt | Senior Staff Fellow | LMSF, NIAID |
| | T. M. Brown | Microbiologist | LMSF, NIAID |
| | M. D. Corwin | Bio. Lab. Tech. | LPB, NIAID |
| | S. F. Hayes | Bio. Lab. Tech. | LPB, NIAID |

COOPERATING UNITS (if any)

Dr. Janne G. Cannon, Dept. of Microbiol. & Immunol., Univ. NC at Chapel Hill, NC;
Dr. David C. Morrison, Dept. of Microbiol., Univ. KS, Kansas City, KS;
Dr. William M. Shafer, Dept. of Microbiol. & Immunol., Emory Univ., Atlanta, GA

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.25 | 2.0 | 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The outer membrane of gram-negative bacteria is comprised of a mosaic of tightly associated lipopolysaccharide (LPS) and proteins. In some organisms, the outer membrane is covered by loosely bound polymers, the bacterial capsule. Since it is the surface of the bacterium which interacts with the host in early stages of parasitism and since it is host defenses directed towards surface constituents which play a role in the prevention and/or resolution of bacterial infection, we are engaged in comparative studies of the structure and function of these outer membrane constituents in three venereal pathogens--Neisseria gonorrhoeae, Chlamydia trachomatis and Contagious Equine Metritis Organism (CEMO). Studies of N. gonorrhoeae include those of LPS, LPS/protein I complexes, H8 antigen (a surface-exposed lipid-protein complex), and capsule (a large molecular weight carbohydrate polymer); studies of C. trachomatis include those of the LPS; whereas the LPS and capsule of CEMO are being investigated. Gonococcal LPS lacks O side chains but is remarkably heterogeneous both structurally and antigenically vis-a-vis the core oligosaccharide. Gonococcal LPS is closely associated with the major outer membrane porin protein (P.I.). This association is impervious to anionic detergents at high temperatures. Immunization with P.I. antigen elicits antibodies to P.I. and LPS. The gonococcal H8 antigen is an abundant, immunogenic surface-exposed antigen common to all strains of pathogenic neisseria. Although the existence of a gonococcal capsule has been the subject of great debate for decades, we have recently obtained morphological evidence (SEM and TEM) of such a capsule. Concurrently carbohydrate polymer has been isolated from cultures of gonococci and is presently being analyzed. The rough LPS of C. trachomatis has a lipid A backbone similar to that of enteric lipid A. Unique long-chain fatty acids, phosphorous, KDO and glucose are also present in this nontoxic LPS. The LPS of CEMO is also rough; some colonial morphotypes produce a capsule which is antigenic and immunogenic in the experimentally infected horse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00236-03 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Studies on the Biology of Contagious Equine Metritis Organism

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. J. Hitchcock Senior Staff Fellow LMSF, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project has been combined with Project No. Z01 AI 00235-04 LMSF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00239-03 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Gonococcal Surface Proteins' Immunochemical Characteristics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. Swanson Chief LMSF, NIAID

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been combined with Project No. Z01 AI 00193-06 LMSF.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00362-02 LMSF |
|---|---|

PERIOD COVERED
October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Primary Structural Analysis of Bacterial Membrane Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | P. A. Barstad | Senior Staff Fellow | LMSF, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
P. A. Barstad transferred to Gull Laboratories, Salt Lake City, Utah, in September 1985, therefore this project is terminated.



PHS 6040 (Rev 1/84) GPO 904 917

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00412-02 LMSF |
|---|---|

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Analysis of the Lyme Disease Spirochete

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | T. R. Howe | Staff Fellow | LMSF, NIAID |
| Other: | A. G. Barbour | Medical Officer | LBP, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.0 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have initiated a molecular approach towards understanding the basic biology and pathogenesis of the Lyme disease spirochete, Borrelia burgdorferi. Having cloned the genes for two major outer membrane proteins of the type strain, B31, on a single recombinant plasmid, we examined the organization of these genes on the original recombinant and in representative clinical and environmental isolates. Characterization of the recombinant plasmid has revealed that the genes for both of these abundant surface proteins are located in the right-most third of the spirochetal insert DNA and are transcribed in the same direction. Transposon Tn5 insertions within the region encoding OspA exert a polar effect on expression of OspB, indicating that these genes share a common promoter. DNA hybridization probes specific for Osp A and B of the type strain, B31 have been prepared and used to examine the organization of these genes in several independent isolates of the Lyme disease borrelia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00413-02 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Chlamydia trachomatis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | F. E. Nano | Staff Fellow | LMSF, NIAID |
| Others: | H. D. Caldwell | Research Microbiologist | LMSF, NIAID |
| | N. G. Watkins | Staff Fellow | LMSF, NIAID |
| | T. Hackstadt | Senior Staff Fellow | LMSF, NIAID |
| | T. Joseph | Graduate Student | LMSF, NIAID |

COOPERATING UNITS (if any)

Hugh Taylor, Johns Hopkins University School of Medicine, Baltimore, MD

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.9 | 1.15 | 1.75 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

As an approach to understanding chlamydial-host cell interactions and the immunobiology of Chlamydia infections, I have begun the molecular cloning of chlamydial antigens. The screening of genomic banks has been done primarily with immunological reagents developed by H. Caldwell. Numerous recombinant clones have been recovered that express surface antigens of Chlamydia trachomatis and C. psittaci. Two sets of recombinant clones--those expressing portions of the major outer membrane protein (MOMP) of C. trachomatis and those expressing the chlamydial genus-specific lipopolysaccharide (LPS) epitope--have been studied in some detail. We have found that the MOMP gene has unusual expression properties in Escherichia coli and that the chlamydial genus-specific LPS epitope is expressed on the surface of enteric bacteria harboring the LPS recombinant plasmids. Expression of the genus-specific epitope in various LPS chemotypes has allowed us to surmise the nature of the epitope. Initial studies in a primate model system indicate that oral vaccination with recombinants expressing the genus-specific epitope provides some protection against subsequent chlamydial disease. I have also cloned genes from C. trachomatis and C. psittaci that encode proteins that bind eucaryotic cell surface components and are present on the infectious form of Chlamydia but are not present on the noninfectious form.



PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00441-01 LMSF

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cloning and Expression of Genes of Rickettsia rickettsii in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. A. McDonald | Staff Fellow | LMSF, NIAID |
| Others: | R. L. Anacker | Research Microbiologist | LMSF, NIAID |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genomic DNA purified from the R strain of Rickettsia rickettsii was cloned into plasmid and bacteriophage cloning vectors. Escherichia coli cells harboring either recombinant plasmids or infected with recombinant phage are screened for the production of rickettsial antigens by their reactivity with polyclonal sera raised against intact R. rickettsii. Thus far, three recombinant plasmids and one recombinant phage, which encode rickettsial antigens, have been identified. The insert from one of the recombinant plasmids was subcloned into pUC8 and pUC9. Cells harboring these plasmids have been used as vaccines in mice in efforts to prevent the death of mice caused by intravenous injection with viable R. rickettsii. Cells harboring the pUC8 subclone offered some protection as 50% of the mice vaccinated in this group survived challenge with a 2 $LD_{50}$ of R. rickettsii.



PHS 6040 (Rev. 1 84) GPO 904-917

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00442-01 LMSF |
|---|---|

PERIOD COVERED
October 1, 1984, to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunobiology of Guinea Pig Inclusion Conjunctivitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | N. G. Watkins | Staff Fellow | LMSF, NIAID |
| Others: | H. D. Caldwell | Research Microbiologist | LMSF, NIAID |
| | F. E. Nano | Staff Fellow | LMSF, NIAID |
| | W. J. Hadlow | Research Veterinarian | LPB, NIAID |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.75 | 1.15 | 0.6 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathogenesis of recurrent ocular chlamydial infections is thought to be immunologically mediated (i.e., hypersensitivity). However, due to the lack of an appropriate animal model of ocular chlamydial infections, the immunological response to chlamydial antigens during infection and challenge has not been characterized. We have chosen to study the immunopathology of guinea pig inclusion conjunctivitis (GPIC), a *Chlamydia psittaci* strain which produces ocular and genital infections in guinea pigs. We have characterized GPIC primary infection clinically and are in the process of characterizing the histopathology of the conjunctiva during primary infection. In addition, we have characterized a delayed type hypersensitivity response to high doses of viable GPIC and to a Triton X-100 extract of GPIC. GPIC recombinant clones producing cutaneous hypersensitivity in immune animals are being characterized to identify the chlamydial antigen which elicits the hypersensitivity response. The immunodominant antigens during primary infection and following challenge have been identified and recombinant clones expressing these proteins have been isolated.



PHS 6040 (Rev. 1/84)

LABORATORY OF PATHOBIOLOGY
Rocky Mountain Laboratories
Hamilton, Montana
1985 Annual Report
Table of Contents

ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1984, TO SEPTEMBER 30, 1985

The Laboratory of Pathobiology was established in January, 1985, in response to the most recent scientific counselors recommendation to assemble a group of scientists able to provide a broader and more modern approach to the important biological questions being addressed by the Epidemiology Branch at Rocky Mountain Laboratories. The present makeup of the laboratory provides a unique and innovative mix of basic biology, biochemistry, immunology, electron microscopy and molecular biology with strong interaction among its own members and with other units both inside and outside of the NIH system. The focus of the laboratory is clearly, however, on the use of molecular biology and modern recombinant DNA procedures to provide a detailed, molecular characterization of host-pathogen relationships. An important by-product of these studies is the potential to produce safe and effective vaccines using well defined, pure, perhaps specifically synthesized immunogenic microbial products. The Laboratory of Pathobiology consists of four sections which describe broad areas of interest. Dr. Claude Garon serves as Acting Chief.

The Molecular Pathobiology Section, a new laboratory set up by Dr. Jerry Keith, is working to characterize in detail the genetic and molecular structure of pathogens with the aim of defining the role of gene products in pathogenic mechanisms. Specifically the section is focused on molecular cloning and expression of genes relevant to the toxic components of Bordetella pertussis. The pertussis toxin has been fractionated into subunits using high pressure liquid chromatography, two of the subunits have been partially sequenced and, based on this amino acid sequence, oligonucleotide DNA probes were synthesized. The DNA probes were used to screen cloned fragments of the pertussis genome DNA. A fragment specific for pertussis toxin has been identified and one of the toxin genes sequenced. Computer analyses of the DNA sequence reveal the molecular structure of the transcriptional unit and identify the location of several regulator sites. A cloned library of pertussis DNA fragments is presently being tested in direct expression vector systems using monoclonal antibodies prepared in this laboratory (KEITH, MUNOZ, LOCHT).

The Arthropod-borne Diseases Section, headed by Dr. Alan Barbour, serves as the principal NIAID laboratory for the study of arthropod-borne diseases such as those caused by tick-borne spirochetes, rickettsiae and viruses. Current work in the section concentrates on two tick-borne spirochetes: Borrelia hermsii, an agent of relapsing fever, and Borrelia burgdorferi, the agent of Lyme disease. Studies of relapsing fever and the antigenic variation of the borrelia have demonstrated that the spirochetes have abundant surface protein that differ markedly in their primary structure at various stages of the infection, thereby avoiding immune clearance by the host. Furthermore, the differential expression of the genes encoding these variable antigens appears related to detectable DNA rearrangements and the duplicative transposition of an antigen-specifying gene from a transcriptionally silent storage site to an active expression site in the

chromosome. These findings have implications for understanding not only relapsing fever, but also other human pathogens that undergo antigenic variation in their hosts (BARBOUR).

The natural history of other arthropod-borne diseases is being investigated by members of this section as well. The international reputation of Dr. Willy Burgdorfer has guaranteed a rich source of field isolated material for study in the laboratory. To evaluate ticks as potential vectors of newly discovered pathogens, laboratory-reared larvae are experimentally infected by allowing them to feed on experimentally infected, septicemic host animals. These together with field-collected ticks are examined by the hemolymph test, by dissection and microscopic examination using direct or indirect immunofluorescence for their ability to serve as effective vectors of disease. Characterization of isolates may include serological and biochemical procedures as well as DNA base composition and SDS-PAGE analyses of protein profiles. Fifteen of 250 D. variabilis from North Carolina and 5 of 100 D. variabilis from Indiana proved infected with rickettsiae that were identified as R. montana. As yet no evidence of R. rickettsii has been found in these selected foci even though human spotted fever has been reported there. The lone-star tick, A. americanum, and the black-legged deer tick, I. scapularis were shown to become infected with the Lyme disease spirochete, Borrelia burgdorferi and may be responsible for the sporatic occurrence of Lyme disease in Texas, Arkansas, Georgia and Tennessee. During the course of investigations related to B. burgdorferi in ticks from California, a borrelia-like spirochete was detected in all three parasitic stages of O. coriaceus, the tick implicated in Epizootic Bovine Abortion (BURGDORFER).

The Immunopathology Section, with Dr. John Munoz as Acting Head, is involved in studies on the immunopotentiating actions of crystalline pertussis toxin. In nanogram doses, pertussigen enhances production of the IgE class of antibodies, promotes induction of experimental allergic encephalomyelitis, enhances delayed-type hypersensitivity and increases inflammation caused by immunological reaction. The toxin is dissociable into five peptides. The exact function of these peptides is unknown except for the ADP-ribosyltransferase activity of the largest peptide. Monoclonal antibodies have been produced in this laboratory and are being tested for specificity against the subunits (MUNOZ).

The Pathobiology Section (formerly Electron Microscopy Section) was established in 1981 and moved into its newly renovated laboratory space in July 1982. The section is responsible for research and research collaboration utilizing modern methods of transmission and scanning electron microscopy as well as other techniques to define those structural alterations that are related to the pathological condition. Techniques employed include, but are not limited to, molecular cloning, nucleic acid microscopy, DNA hybridization, electron immuno-microscopy, ultramicrotomy, freeze etching and other methods allowing a full range of morphological evaluation. While several classes of viruses form stable associations with their hosts by integrating one or more copies of their genomes into the host cell DNA, retroviruses provide a unique and important system for the study of integrative recombination. For that reason molecular clones of several newly integrated retroviral genomes were produced in either bacteriophage or plasmid cloning vehicles using recombinant DNA techniques and were characterized by heteroduplex and/or R-loop methods. These studies have

not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells. Unique inverted repeat sequences structurally resembling bacterial transposable elements have been identified in human cell DNA, isolated and molecularly cloned. (GARON).

Guest researchers in the laboratory this past year have included: Stanley Falkow (Stanford University School of Medicine), Richard Sherburne (University of Alberta, Canada), Kenneth Gage (University of Oklahoma), Robert Lane (University of California, Berkeley) Kirsten Vadheim (Montana State University). Departures due to retirement or transfer have included: Aftab Ansari, Leo Thomas, Ann Leatherbury and Sandy Tessier. In June 1985, the Laboratory of Pathobiology, along with other laboratories at RML were reviewed by the NIAID Board of Scientific Counselors.

ANNUAL REPORT
LABORATORY OF PATHOBIOLOGY
ROCKY MOUNTAIN LABORATORIES
HAMILTON, MONTANA
NATIONAL INSTITUTES OF ALLERGY AND INFECTIOUS DISEASES
OCTOBER 1, 1984, TO SEPTEMBER 30, 1985

## HONORS AND AWARDS

Journal Editorial Boards:

W. Burgdorfer - Acta Tropica; Co-Editor of Current Topics in Pathogen-Vector-Host Research; Journal of Medical Entomology

W. Hadlow - Member of Editorial Board for Fundamental and Applied Toxicology
Reviewed book for American Scientist "Scrapie Disease in Sheep" by H. B. Parry

Manuscripts from J. Wildlife Diseases, Veterinary Pathology, Fundamental and Applied Toxicology, Science, Infection and Immunity, Proceedings of National Academy of Sciences, American Journal of Medical Hygiene, Infectious Disease, and Proceedings of Experimental Biology and Medicine were also reviewed by members of LPB staff.

Professional posts:

A. Barbour - Elected Fellow, Infectious Disease Society of America

W. Hadlow - Continued as Adjunct Professor of Veterinary Pathology, Washington State University, Pullman, WA
Member of Education Committee of the American College of Veterinary Pathologists

J. Munoz - Reappointed as a Staff Affiliate of the University of Montana
Judge, Montana State Science Fair, Missoula, MT
Trustee for the Stella Duncan Research Fund, University of Montana, Missoula, MT

Invited Lectures and Participation in Meetings and Symposia:

C. Garon - Invited to contribute a chapter to "Ultrastructure Techniques for Microorganisms"

A. Barbour - Spirochete Symposium, I.C.A.A.C., A.S.M., Washington, D.C.
"Molecular Biology of Bacterial Pathogens," American Society for Microbiology, Las Vegas, NV
"DNA Rearrangement: Process and Purpose," American Society for Microbiology, Las Vegas, NV
University of Texas, Department of Microbiology, San Antonio, TX
California Institute of Technology, Pasadena, CA
Department of Microbiology, Montana State University, Bozeman, MT

Medical Grand Rounds, University of Utah School of Medicine, Salt Lake City, UT
Invited as co-convener and chairman at the Second International Lyme Disease Symposium, Vienna, Austria

W. Burgdorfer - Presented lecture "Zue Entdeckung der Lyme disease (Erythema chronicum migrans) Spirochate" at the 34th Annual Meeting of the German Dermatology Society, Zurich, Switzerland.
Lyme Disease - an enlarging spectrum, presented at the Annual Meeting of the Connecticut Valley Branch of ASM, Groton, CT.
Presented the Benjamin M. King Memorial Lecture "Tick/Spirochete Relationship in Lyme Disease" at the spring meeting of the South Central Association for Clinical Microbiology, Grand Rapids, MI.
Presented at the University of Neuchatel, Switzerland, a series of lectures on Rickettsiae and Rickettsial Diseases as part of an advanced parasitology course.
Participated and presented two research papers at the Workshop on Luekocytic Rickettsiae of Man and Animals, University of Illinois, Urbana-Champaign, IL.
Invited to present R. R. Parker Memorial Lecture at the 40th Annual Conference on Diseases in Nature Communicable to Man, Vancouver, British Columbia, Canada.
Invited to present "From Bench to Bedside - The Discovery of Lyme Arthritis Pathogen" at the 49th Montana Scientific Meeting of The Montana Society of Internal Medicine at Fairmont Hot Springs, MT.
Invited to serve as co-convener and chairman at the Second International Symposium on Lyme Disease and Related Disorders, Vienna, Austria. Will present two research papers.
By invitation, wrote chapter on "Lyme Disease" for the Second Edition of Oxford Textbook of Medicine.

W. Hadlow - Presented lecture "Slow Viral Diseases: at the Ninth Annual Davis Foundation Symposium on Veterinary Pathology, Ames, IA.

J. Munoz - Invited to participate at a symposium to be held at the University of Washington, Seattle, WA.
Gave a seminar on "Pertussis Vaccine. Risks and Benefits" at the University of Montana, Missoula, MT.

OTHER HONORS AND AWARDS:

W. Burgdorfer - Received the Schaudinn-Hoffman Award for the discovery of the Lyme disease spirochete, *Borrelia burgdorferi* during the 34th Annual Meeting of the German Society of Dermatology in Zurich, Switzerland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00061-23 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Natural History of Tick-borne Rickettsiae and Their Public Health Significance

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI. | Willy Burgdorfer | Research Entomologist (Med) | LPB, NIAID |
| OTHERS: | L. A. Thomas | Research Microbiologist | LPB, NIAID (Retired) |
| | M. G. Peacock | Microbiologist | LPB, NIAID |
| | R. A. Anacker | Research Microbiologist | LMSF, NIAID |

COOPERATING UNITS (*if any*) Univ. Georgia, Athens (C. Greene), Sion, Switzerland (O. Peter), Univ. Ill. (C. J. Holland), Ball State Univ. (R. R. Pinger), Univ. Neuchatel, Switzerland (A. Aeschlimann)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.4 | 0.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project concerns studies of Rocky Mountain spotted fever and other tick-borne rickettsial diseases in the United States and in certain other countries with emphasis on ecology, identification, and characterization of rickettsiae and their relationship(s) to the respective tick vectors. Source material is obtained through collaboration with outside agencies. The project also considers the cellular and subcellular aspects of interactions between tick-borne rickettsiae and their vectors, particularly the mechanism(s) of interference and the factors responsible for changes in the agent's pathogenicity. Isolation of rickettsiae are made from infected ticks or from bloods of patients or animals by injection into susceptible animals or cell cultures. Characterization of isolates includes serological and biochemical methods (microagglutination, microimmunofluorescence, DNA base composition and protein (SDS-PAGE) determinations). Interactions between rickettsiae and their arthropod vectors is followed by light, fluorescence and electron microscopy of tissues from naturally or experimentally infected ticks. Tissue cultures are being used to study mechanisms of rickettsial development and infection in host cells. Rocky Mountain spotted fever and ehrlichiosis in dogs, although difficult to differentiate clinically, can readily be diagnosed by specific immunofluorescence. The newly detected etiologic agent of Potomac Horse fever is a rickettsia of the genus Ehrlichia. The name E. risticii nov. sp. has been proposed. Although the modes of transmission of this agent has not as yet been established, ticks are considered as possible vectors. Rhipicephalus sanguineus and Dermacentor variabilis have been shown to maintain this agent. Attempts to isolate R. rickettsii from D. variabilis collected in spotted fever areas of North Carolina and Indiana have so far been negative. The only rickettsial agent recovered is R. montana. Monoclonal antibodies to R. rickettsii are being evaluated for rapid identification of ticks infected with this agent.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00063-15 LP

PERIOD COVERED
October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immune Responses to Rickettsial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | M. G. Peacock | Microbiologist | LPB, NIAID |
| OTHERS: | L. A. Thomas | Research Microbiologist | LPB, NIAID (Retired) |
| | W. Burgdorfer | Research Entomologist (Med) | LPB, NIAID |
| | D. W. Hackstadt | Senior Staff Fellow | LMSF, NIAID |
| | S. F. Hayes | Biol. Lab. Tech. | LPB, NIAID |

COOPERATING UNITS (if any) USARMIID, Frederick, MD (J. C. Williams), Sion, Switzerland (O. Peter), Orlando Regional Medical Center, Florida (R. G. Brooks)

LAB/BRANCH
Laboratory of Pathobiology

SECTION
Arthropod-borne Diseases Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 0.4 | 0.8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study immune responses in man and animals to natural and experimental rickettsial infections, particularly Q fever, and to isolate and identify the causative pathogens. For serological investigations, recently developed procedures such as indirect immunofluorescence, microagglutination,and the enzyme-linked immunosorbent assays (ELISA) are being used. It also provides serologic support to other RML units and occasionally also to outside agencies, and includes serodiagnosis of other bacterial or viral diseases under investigation. For the isolation of pathogens, susceptible laboratory animals (meadow voles, guinea pigs, embryonated hen eggs, etc.) and various tissue culture systems (Vero, L cells, etc.) are being used. Serologic tests as well as immunochemical procedures (SDS-PAGE, western blotting) are applied to identification of isolates. The indirect IF test proved to be a valuable tool in early diagnosis of Q fever during a large outbreak in Switzerland. In spite of antibiotic therapy, Coxiella burnetii was found to destroy the aortic or mitral valves in patients with chronic Q fever endocarditis. It similarly affects the porcine valve replacement and causes detachment of the inserted plastic prosthesis. An unusual case of chronic Q fever endocarditis with neurological abnormalities was serologically diagnosed. After 25 years storage in aqueous suspension at 4°C, the experimental RML Q fever vaccine (Q 58 A) was found to have retained its initial antigenic potency. Results of animal tests suggest that this vaccine also protects against C. burnetii from Q fever endocarditis patients.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00071-14 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Pertussigen

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | |
|---|---|---|
| John J. Munoz, PI | Research Microbiologist | LPB, NIAID |
| Jerry M. Keith | Senior Staff Fellow | LPB, NIAID |
| Kevin Marchitto | Senior Staff Fellow | LPB, NIAID |
| Camille Locht | Visiting Fellow | LPB, NIAID |

COOPERATING UNITS (if any) Dr. Elmer Becker, Dept. of Pathology, University of Connecticut, Farmington, CT.

LAB/BRANCH

Laboratory of Pathobiology, RML, Hamilton, MT 59840

SECTION

Immunobiology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | .8 | .2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Pertussigen (pertussis toxin) (Ptx) is the toxin from Bordetella pertussis responsible for most of the biological activities known for pertussis vaccines. In nanogram doses Ptx enhances production of IgE class of antibodies, promoted induction of experimental allergic encephalomyelitis, enhances delayed type of hypersensitivity and increases inflammation caused by immunological reaction. Ptx also increases production of insulin, increases susceptibility to histamine and other vasoactive substances, induced lymphocytosis and has many other actions of interest. Our main aims are to elucidate the mode of action of Ptx and to develop a non-toxic effective vaccine for whooping cough. Ptx is composed of five peptides (S-1, S-2, S-3, S-4, and S-5) of which the S-1 is known to have an ADP-ribosyl transferase activity. Little is known about the function of the other subunits. Our efforts this year have been channeled toward the development of specific monoclonal reagents to detect each of these subunits. We now have specific monoclonals for S-1, S-2 and S-4. With these reagents, it will be possible to study the role each peptide plays in the biological activities of Ptx.

Since Ptx is most likely the main protective antigen in pertussis vaccine, we are investigating the possibility of developing by genetic engineering techniques a Ptx molecule that lacks toxicity but still can protect against pertussis.

In collaboration with Dr. Elmer L. Becker of the University of Connecticut, we have shown that Ptx inhibits neutrophil granule enzyme secretion and the chemotactic response to formylmethionyl-leucyl-phenylalanine (fMet-Leu-Phe).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 0082-24 LP

PERIOD COVERED

October 1, 1984, to September 20, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Relation of Viruses to the Genesis of Chronic Disease

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: W. J. Hadlow Research Veterinarian (Pathology) LPB, NIAID

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

To obtain an insight into the unusual host-virus interactions resulting in slowly evolving diseases, two natural viral infections of domestic animals are studied by simple methods of clinical observation, animal inoculation, serology, virology, and anatomic pathology. These diseases are (1) scrapie of sheep and goats and (2) Aleutian disease of ranch mink. Scrapie is a degenerative disease of the brain caused by an unconventional virus. Replication of the virus in central nervous tissue, which gives rise to the slowly progressive polioencephalopathy, is preceded by many months of replication in extraneural sites, notably lymphoreticular tissues and intestine. Observations on naturally infected lambs and experimentally inoculated fetal and newborn Suffolk sheep provided little information on the early events in the infectious process, especially those events that might bear on modes of natural transmission. This was so because of the long period between exposure to virus and its first detection by mouse inoculation, the only practical way for doing so. The lack of a more suitable detection method and the absence of an immune response to the infection continue to hamper study of this unusual infectious disease. Aleutian disease, caused by a parvovirus, is a chronic renal disease brought about by circulating virus-antibody complexes that become deposited in the glomeruli. Aleutian and non-Aleutian mink were found equally susceptible to infection with several strains of virus. The infection gave rise to viremia in all Aleutian mink but in only some non-Aleutian mink, Disease did not supervene, however, unless the viremia persisted beyond the first few months after exposure to virus. These findings emphasize the need to distinguish between infection and disease when efforts are made to understand the pathogenesis and epidemiology of Aleutian disease. Information obtained from these studies has implications for understanding comparable protracted human diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00201-06 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Integrated Viral Genomes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | Claude F. Garon | Acting Chief | LPB, NIAID |
| OTHERS: | Lori L. Jansen | Biologist | LPB, NIAID |

COOPERATING UNITS (if any) LMO/NCI (T. S. Papas), FDA (R. P. Silver)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Pathobiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.8 | 0.8 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

While several classes of viruses form stable associations with their hosts by integrating one or more copies of their genomes into the host cell DNA, retroviruses provide a unique and important system for the study of integrative recombination. Retroviral genomes are integrated with high efficiency at specific sites within the viral genome, but at a large number of sites in the host chromosome. Often a consequence of this integration event is a readily detectable change in cell growth. Modern methods of molecular cloning and analysis allow for the detection and amplification of rare DNA sequences such as an integrated viral segment. Molecular clones of several newly integrated retroviral genomes were produced in either plasmid or bacteriophage cloning vehicles using recombinant DNA techniques and were characterized using electron microscope heteroduplex and R-loop methods. Detection of sequence homology even when interrupted by intervening cellular DNA is often accurately mappable in the electron microscope using these methods. These studies have not only shown the arrangement of integrated viral sequences within infected host cell DNA, but have also demonstrated the presence and sequence arrangement of certain viral transforming sequences within normal, uninfected host cells as well. Unique inverted repeat sequences structurally resembling bacterial transposable elements have been identified and molecularly cloned. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of integrative position or flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombinaton in eukaryotic cell systems.



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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00231-04 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Biology of Pathogenic Borreliae and Borrelial Infections

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | Alan Barbour | Chief, Section | LPB, NIAID |
| OTHERS: | Paul Barstad | Senior Staff Fellow | LMSF, NIAID |
| | Sven Bergstrom | Visiting Fellow | LMSF, NIAID |
| | Willy Burgdorfer | Research Entomologist | LPB, NIAID |
| | John Coligan | Senior Scientist | LIG, NIAID |
| | Claude Garon | Acting Lab Chief | LPB, NIAID |
| | Timothy Howe | Staff Fellow | LMSF, NIAID |
| | Joseph Meier | Graduate Student | LPB, NIAID |

COOPERATING UNITS (*if any*) State U. of NY at Stony Brook (J. Benach), U. of Calif., Berkeley (R. Lane), Calif. Institute of Technology (R. Plasterk, M. Simon), Karolinska Institute, Stockholm (B. Skoldenberg), Univ. of Vienna (G. Stanek), Yale Univ. (A. Steere), and Univ. of Munich (B. Wilske)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.1 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Arthropod-borne members of the genus Borrelia cause relapsing fever and Lyme disease. We are taking biochemical and molecular biological approaches to the study of pathogenesis of these human disorders. With regard to the antigenic variation in relapsing fever, we have identified and characterized the variable antigens and are establishing the molecular genetic basis for the variation. In our studies of Lyme disease, we identified several antigenic components of the etiologic spirochete, developed monoclonal antibodies to these components, and cloned genes for surface antigenic proteins into _E. coli_.



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00232-04 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Lyme Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Alan G. Barbour

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with project Z01 AI 00231-04 (Biology of Pathogenic Borreliae and Borrelial Infections).



PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00268-04 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ecology of Lyme disease and related disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


| | | | |
|---|---|---|---|
| PI: | W. Burgdorfer | Res. Entomologist (MED) | LPB, NIAID |
| OTHERS: | A. G. Barbour | Sr. Staff Fellow | LMSF, NIAID |
| | S. F. Hayes | Biol. Lab. Tech. | LPB, NIAID |


COOPERATING UNITS (if any) Univ. Calif, Berkeley (R. S. Lane), Minn. Dept. of Health, Minneapolis (M. T. Osterholm), Univ. Conn. Health Center, Farmington (L. Reik), Munich, W. Germany (K. Weber), Univ. Neuchatel, Switzerland (A. Aeschlimann) Univ. Minnesota (R. C. Johnson)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | .6 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The purpose of this project is to determine the natural history of the recently discovered and isolated causative agent of Lyme disease and related disorders. The relationship(s) between the spirochete and its various tick vectors (Ixodes dammini, I. pacificus, I. ricinus, Amblyomma americanum) is being determined by establishing through conventional as well as transmission and scanning electron microscopy (a) the development of the spirochete within the ticks, and (b) the mode(s) of transmission to vertebrate hosts. In cooperation with outside agencies, tick/spirochete surveys are being conducted to determine prevalence of infected ticks in endemic foci. Similarly, the natural source(s) for infecting ticks is being evaluated first serologically (indirect immunofluorescence) and subsequently through recovery of spirochetes from serologically implicated hosts. The western deer tick, Ixodes pacificus, has been confirmed as the vector of Borrelia burgdorferi in western U.S. Of 1,647 adult ticks from California and Oregon, 25 (1.4%) contained spirochetes indistinguishable from the Lyme disease agent. The lone star tick, Amblyomma americanum and the black-legged deer tick Ixodes scapularis, have been shown experimentally to maintain and transmit B. burgdorferi; these ticks must be considered potential vectors in southern and southeastern U.S. The cotton rat (Sigmodon hispidus) appears to be susceptible to the Lyme disease spirochete and experiences spirochetemias typical for relapsing fever spirochetes. For patients with neurological abnormalities (aseptic meningitis, encephalitis, neuritis, radiculitis, etc.) Lyme disease should be considered in a differential diagnosis. A hitherto undescribed borrelialike spirochete was detected in the argasid tick, Ornithodoros coriaceus and is considered a potential cause of epizootic bovine abortion (EBA).




PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00402-02 LP

PERIOD COVERED

October 1, 1984, to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Cloning and Expression of *Bordetella pertussis* Toxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. M. Keith | Acting Section Chief | LPB, NIAID |
| Others: | C. Locht | Visiting Fellow | LPB, NIAID |
| | K. S. Marchitto | Sr. Staff Fellow | LPB, NIAID |
| | S. G. Smith | Microbiologist | LPB, NIAID |
| | K. Vadheim | Guest Worker | LPB, NIAID |
| | J. J. Munoz | Research Microbiologist | LPB, NIAID |
| | S. E. Coligan | Senior Scientist | LIG, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Pathobiology

SECTION

Molecular Pathobiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.65 | 2.4 | 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The Molecular Pathobiology Section's functional objectives are to investigate the genetic molecular structure of pathogens, to define the role of gene products in pathogenic mechanisms and to perform studies directed toward development of vaccines using molecular or synthetic production of immunogenic peptides from microbial agents. Our major emphasis is focused on cloning and expression of genes relevant to the toxic components of *Bordetella pertussis* i.e., the bacteria responsible for whooping cough. Our immediate goal is to detoxify pertussis by molecular manipulation of the genome, thus producing a "cleaner bug" for use in second generation vaccine development. Using monoclonal antibodies, we are currently working on the expression of pertussis toxin i.e., lymphocytosis promoting factor, which we have cloned and sequenced. Our long term interests are in the identification and understanding of epitopes which stimulate synthesis of protective antibodies and the development of third generation vaccines such as protein subunits and synthetic peptide antigens.

Development of a safer "new generation" pertussis component vaccine using molecular cloning of *Bordetella pertussis* genes has been hampered by the inherent difficulties in identifying DNA fragments containing specific pertussis genes for protective antigens. Cloning strategies which rely expression of protein in *E. coli* have not been very successful probably because of differences in the gene regulation transcriptional signals. Alternate more direct strategies using oligonucleotide probes coding for specific portions of proteins may be more successful. The polycistronic nature of prokaryotes suggest that the genes coding for the protein subunits of pertussis toxin may be in a tandem arrangement regulated by one operator. Recently, we have cloned and partially sequenced a 4.2 kb EcoRl/BamHl DNA fragment containing at least two of the five subunit genes of pertussis toxin.




PHS 6040 (Rev. 1/84) GPO 914-918

Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
1985 Annual Report
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Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

RESEARCH HIGHLIGHTS

Mouse scrapie prion protein (PrP) gene cloned. This gene was expressed as mRNA in brain tissue of both scrapie-infected and uninfected mice and hamsters. The prion protein appears to be a normal CNS protein. It is hypothesized that its accumulation in an aggregated fibril form in scrapie brain is secondary to tissue destruction induced by the scrapie agent.

Retrovirus-induced immunosuppression in the Friend murine leukemia virus system was influenced by genes within the H-2 complex of mice. This effect of H-2 was independent of the H-2 effect on recovery from Friend virus leukemia.

Neurotropic wild mouse ecotropic retrovirus was observed to replicate in the spinal cord of mice both sensitive and resistant to the clinical disease. Thus the mechanism(s) of resistance to this disease appeared not to function via control of CNS spread or replication.

Recombination of murine retroviruses with endogenous viral gene sequences was shown to be specified by the 3' LTR region of the exogenous viruses inoculated.

Polytropic recombinant retroviruses were demonstrated in preleukemic AKR mice as early as 1 month of age. The role of these viruses in leukemogenesis is now under investigation.

ts mutant of avian MH2 retrovirus was found to have an alteration in the myc gene, which affected macrophage transformation without affecting fibroblast transformation.

Hemolytic anemia induced by murine retroviruses. Rapid splenomegaly induced by certain strains of Friend murine leukemia helper virus was due to splenic hematopoiesis secondary to virus-induced hemolysis. Hemolytic anemia, rather than leukemia, appeared to account for death of most mice inoculated with these viruses.

Sites of in vivo Aleutian disease virus sequestration were identified in mink tissues by in situ hybridization and reactions with monoclonal antibodies. Cells replicating virus appeared to be a minority of the virus-positive cells observed.

Rapid immunity to CNS challenge of rabies virus was observed in SJL mice as soon as 5 days after primary intraperitoneal inoculation of live virus. These data demonstrate the existence of potent antiviral immune mechanisms capable of acting against this virus even within the CNS.

Amyloid-female protein interaction. *In vivo* half-life experiments demonstrated that tissue amyloid deposits and serum female protein of hamsters were in a dynamic equilibrium. This suggests the possibility that amyloid deposition could be halted and perhaps even dissolved by agents known to bind female protein.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

ADMINISTRATIVE REPORT

The following staff changes occurred in LPVD in the past year: Dr. Susan Carpenter from the Department of Veterinary Medicine, University of Massachusetts joined the LPVD as a Staff Fellow. Dr. Howard Etlinger, a Senior Staff Fellow, left LPVD to take a position at Hoffman LaRoche in Basel, Switzerland. Dr. Oskar Kaaden from Hanover, Germany arrived to spend a year as a Guest Worker working with Aleutian disease virus of mink.

Summer student guest workers were: Kate Nathanson, Haverford College, Haverford, PA; Swend Holland, Carroll College, Helena, MT; Eric Huggins, Montana State University, Bozeman, MT.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984 to September 30, 1985

## HONORS AND AWARDS

Professional Posts:

Dr. B. Chesebro - Adjunct Professor - Department of Microbiology, Montana State University, Bozeman, MT

Dr. M. E. Bloom - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

Dr. J. E. Coe - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

Dr. D. L. Lodmell - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00074-13 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | B. Chesebro | Chief | LPVD, NIAID |
| Others: | R. Morrison | Staff Fellow | LPVD, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.3 | 0.8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Studies of the mechanisms of genetic control of recovery of adult mice from Friend virus complex (FV)-induced leukemia revealed that the H-2D subregion appeared to influence recovery by altering the kinetics of generation of FV-specific helper T lymphocytes. In addition, gene(s) within the H-2 complex were found to affect the ability of FV to induce immunosuppression to non-retroviral antigens. This resistance to retrovirus-induced immunosuppression occurred in the presence of viremia and persistent leukemic splenomegaly. Furthermore, immunosuppression was not inhibited by the presence of the $Rfv\text{-}3^{r/s}$ genotype which enabled certain leukemic mice to mount an effective humoral immune response to FV. Mice with the $H\text{-}2^{a/a}$ and $Rfv\text{-}3^{r/s}$ genotypes appeared to be similar to AIDS patients in that they made humoral antiviral antibody but were immunosuppressed to challenge with nonviral antigens. Thus, the immune response to viral and nonviral antigens appeared to be influenced by separate host genes in this system. Elucidation of the factors controlling these immune responses should be of value in understanding similar responses in AIDS.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00260-04 LPVD |
|---|---|

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | B. Chesebro | Chief | LPVD, NIAID |
| Others: | M. Sitbon | Visiting Fellow | LPVD, NIAID |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.3 | 0.8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*


A new focal immunofluorescence assay (FIA) was developed for use in assaying and biologically cloning retroviruses infecting live cell monolayers. By using monoclonal antibodies with specificity for only certain strains of murine retrovirus, it was possible to measure interference to superinfection of chronically infected cells. Surprisingly the patterns of interference varied markedly when the same viruses were compared in different cell lines. These results suggested that specificity of cellular receptors for retroviruses could vary widely in different cell lines. Furthermore, the patterns of interference seen in one particular cell line could not be explained by the presence of a small number of different receptor types.

The FIA has also been used to compare virus replication and recombinant MCF virus generation in mice inoculated with a low virulence variant Friend murine leukemia virus strain (F-MuLV-B3). Results indicated that B3 was not defective in replication or recombinant MCF virus generation, nevertheless in vivo leukemic transformation as detected by splenomegaly or lymphadenopathy was delayed by several months compared to the parental virus strain (F-MuLV 57). Preliminary results suggest that strains B3 and 57 may not differ in ability to transform hemopoietic cells, but rather in their ability to induce hemolytic anemia with compensatory splenomegaly early after inoculation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00072-14 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Host and Viral Factors in Resistance to Rabies Virus Infection in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | D. L. Lodmell | Scientist Director | LPVD, NIAID |
| Others: | None | | |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Murine resistance to intraperitoneally (i.p.)-inoculated street rabies virus (SRV) has been shown to be dominant and genetically controlled by the concurrent presence of each of two segregating genes. Trace experiments for infectious SRV indicated that susceptibility differences among genetically dissimilar strains of mice were associated with restriction of viral replication within the central nervous system (CNS). Limitation of viral replication appeared to correlate with the antibody response. The importance of the immune response was reaffirmed with cyclophosphamide studies in that all resistant SJL/J mice died following immunosuppressive treatment. In contrast, cyclophosphamide-treated SJL/J mice and immunodeficient athymic mice were protected when reconstituted with immune serum starting at 72 hr after SRV inoculation, a time in which virus was not detected in the peritoneal cavity, but was present in the spinal cord. Passively transferred unfractionated immune cells also protected athymic mice. Specific cell eliminations with cytotoxic antibody and complement indicated B cells, but not T cells, were essential for protection. Additional studies showed that neutralizing antibody in the cerebrospinal fluid was unimportant in the resistance of mouse strains which remained CNS clinically asymptomatic. Furthermore, the CNS of mice inoculated i.p. 5 days previously with SRV was resistant to either intracerebral or intranasal rabies virus challenge. Survival of these mice correlated with the detection of neutralizing antibody in serum. A focal immunofluorescent assay (FIA) on live cells has been developed for quantification and biological cloning of street and laboratory-adapted strains of rabies viruses. Monoclonal antibodies, in conjunction with mutagenic agents and the FIA assay, are being utilized for selection of avirulent and virulent isolates of rabies viruses. Preliminary studies indicate that only monoclonal antibodies with neutralizing activity inhibit replication of rabies virus <u>in vitro</u>, and protect cyclophosphamide immunosuppressed SJL/J mice.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00261-03 LPVD |
|---|---|

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Immunological Aspects of Neurovirology

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | D. L. Lodmell | Scientist Director | LPVD, NIAID |
| Others: | None | | |

COOPERATING UNITS (*if any*)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| | | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00073-19 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*) Mechanisms of Immunity and Immunopathology Related to Cellular/Humoral Immunity - Coe

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI: J. E. Coe Medical Officer LPVD, NIAID

Others: None

COOPERATING UNITS (*if any*)

None

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Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

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NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

No work has been done on this project during the past year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00262-04 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. E. Coe | Medical Officer | LPVD, NIAID |
| Others: | None | | |

COOPERATING UNITS (if any) Dr. B. Dowton, Harvard Medical School, Boston, MA; Dr. K. Ishak, AFIP, Washington, D.C.; Dr. S. S. Mookerjea, Univ. of Newfoundland, St. Johns, Newfoundland; Dr. U. Nilsson, Univ. Hospitals, Uppsala, Sweden; Dr. J. Sogn, NIAID, Bethesda, MD; Dr. R. Mortensen, Ohio State Univ., Columbus, OH

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

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NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Syrian hamster has a peculiar sex limited serum protein, expressed as a major protein in females (therefore called Female Protein) and testosterone suppressed in males. Female Protein (FP) is a homolog of two human pentraxins, C-reactive protein (CRP) and amyloid P component (AP) as shown by similar structure (pentameric) and amino acid sequence. Furthermore, FP shares function-properties with both human pentraxins such as $Ca^{++}$ dependent phosphorylcholine binding, complement fixation, acute phase responsiveness (characteristics of CRP) and also is a constituent of amyloid (characteristic of AP). Indeed, high serum levels of FP occurring naturally (as in female) or experimentally (as in hormonally treated male) are directly associated with deposition of amyloid. Whether these high serum FP levels are directly responsible for amyloid deposition is unknown at present. However, by following the metabolism of injected $^{125}$I-FP, an extraordinary sequestration of serum $^{125}$-FP can be demonstrated within amyloid deposits. This diagnostic alteration of a serum protein (FP) metabolism in the amyloidotic hamster, has not been observed in other amyloid models. The mechanism is still unknown, although it indicates a dynamic exchange of FP between serum and amyloid compartments. Present experiments are designed to limit and reverse amyloid deposition by control of serum FP levels and alteration of FP binding capacity. Although FP is a major serum component (of females) and represents an ancient protein which has changed little during evolution, its reason for existence is unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00085-08 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)


| | | | |
|---|---|---|---|
| PI: | M. E. Bloom | Medical Officer | LPVD, NIAID |
| Others: | D. L. Wiedbrauk | Staff Fellow | LPVD, NIAID |
| | R. E. Race | Veterinary Officer | LPVD, NIAID |


COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.3 | 0.8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The purpose of this project is the study of Aleutian disease (AD) of mink, a persistent infection by the Aleutian disease parvovirus (ADV). We have extended studies to include in situ hybridization using as hybridization probe radiolabeled molecularly cloned ADV DNA. Replication in cell culture was accompanied by the development of nuclear viral antigen and large numbers of autoradiographic grains over the nuclei of infected cells. This result contrasted markedly with findings made in tissues of infected mink. In spleen and mesenteric lymph node (MLN), ADV DNA was readily detected primarily in the cytoplasm or on the membranes of cells. Furthermore, in MLN grains were found localized to the periphery of germinal centers in a reticular pattern reminiscent of that described for protein antigens following immunization. Since viral antigens had the same distribution in these sections, this suggested that the DNA observed probably represented virus particles sequestered by elements of the immune system rather than sites of virus replication. Rare single cells contained grains localized over the nucleus, and this observation implied that the number of cells actually replicating ADV in these tissues was small. Extensive attempts were made to characterize infected cells by culturing infected infected <u>in vivo</u> or <u>in vivo</u> with ADV. ADV replication could not be convincingly demonstrated although the lymphocytes were stimulated with mitogens and mink T cell growth factor. These results suggested that the target cell for ADV is either not a lymphocyte or that the conditions for its cultivation in <u>vitro</u> are extremely fastidious.

In other studies, the possible role of interferons in the pathogenesis of ADV infections has been begun. ADV infection in cell culture induces interferon at both 31.8°C and 37°C, although the induction at 37°C is much more rapid than that at 31.8°C. Because ADV replicates only at the lower temperature, this finding may suggest a potential role for interferon in the suppression of ADV replication at 37°C.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00263-04 LPVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | M. E. Bloom | Medical Officer | LPVD, NIAID |
| Others: | D. L. Wiedbrauk | Staff Fellow | LPVD, NIAID |
| | D. L. Lechner | Guest Worker | LPVD, NIAID |
| | O. Kaaden | Guest Worker | LPVD, NIAID |

COOPERATING UNITS (*if any*)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.3 | 0.7 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The purpose of this project is the study of genome structure and function of the Aleutian disease parvovirus (ADV). Further studies have included detailed physical mapping of genomic segments from three strains of ADV. These clones were derived by molecular cloning of replicative forms prepared from Hirt supernatants of infected cell cultures. The results indicated that the three viruses were very similar, but that discreet differences could be detected in the portion of the genome coding for viral structural genes. An alternate strategy was employed to study DNA from viruses that do not grow in cell culture. Single stranded virion DNA was prepared from virus purified from the organs of mink infected with either Utah I ADV or Pullman ADV. This DNA was converted to duplex molecules in vitro, cleaved with restriction enzymes and cloned. Molecular clones were derived that expressed viral antigens in *E. coli*. It will now be possible to compare the genomic structure and function of ADV strains without concern for possible selection factors exerted by cell culture adaptation. Detailed analysis of these molecular clones is currently underway. We have also constructed a full length molecular clone of ADV-G, developed by tailing of intact replicative form. Preliminary analysis indicates that DNA from this recombinant plasmid produces viral antigen when transfected into cultures of cells permissive for ADV replication.

Finally, transcription of the ADV genome is also under study and the results to date suggest that ADV encodes at least three mRNA species ranging in size from 2-4 kb. One of these, the 4.0 kb RNA, can be detected prior to the onset of detectable DNA replication. This mRNA may represent the nonvirion protein thought by some to have a role in modulating viral DNA replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00401-01 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Possible Animal Models for the Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | M. E. Bloom | Medical Officer | LPVD, NIAID |
| | R. E. Race | Veterinary Officer | LPVD, NIAID |
| Others: | None | | |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 AI 00086-08 LPVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Mechanisms of Resistance to Graft Versus Host Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. L. Portis | Medical Officer | LPVD, NIAID |
| Others: | B. Chesebro | Chief | LPVD, NIAID |
| | S. Hayes | Bio. Lab. Tech. (Micro.) | LPB |

COOPERATING UNITS (if any)
Dr. Richard Ziegler, Univ. Minnesota, Duluth, MN

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.2 | 1.1 | 1.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has recently been recognized that some human retroviruses are capable of replicating in the central nervous system. Their participation in CNS pathology is as yet a matter of speculation. We are studying a murine retrovirus (WM-E) which was originally isolated from wild mice and has the capacity to cause a lower-motor-neuron paralytic disease in some strains of laboratory mice with a 12-20 week latency. The primary goal of this project is to identify the cells in the CNS which replicate this virus and the mechanisms by which this virus causes motor neuron pathology. We have prepared a panel of monoclonal antibodies specific for WM-E in AKR mice which are tolerant to endogenous retrovirus. These antibodies react with the three viral membrane-associated proteins gp70, p15(E) and $p15^{\underline{gag}}$ and express no cross-reactivity with endogenous viruses of laboratory mice. Using these antibodies to follow virus replication, we have found that neonatal inoculation of both susceptible (NFS) and resistant (AKR, NZB) strains of mice resulted in quantitatively equivalent levels of WM-E virus replication in the spleen and comparable levels of viremia. We have documented that the virus does indeed replicate in the lumbar spinal cord by both immunohistochemistry and infectious center assay of trypsin/collagenase-disrupted tissue. However, no significant difference was found in the levels of virus replication in the lumbar cord of susceptible and resistant strains of mice. In addition, retroviruses which do not cause neurologic disease also replicated in the CNS, but the patterns of replication as detected by EM appear different when compared with that of WM-E. We are currently studying primary cultures of lumbar cord cells in order to identify the specific cell types which are infected by these various viruses. Another retrovirus has now been identified which is unrelated to WM-E, but which also causes paralytic disease in mice. This virus is being molecularly cloned by B. Chesebro and should prove useful in identifying viral genomic sequences responsible for the neuropathology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00264-04 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. L. Portis | Medical Officer | LPVD, NIAID |
| Others: | R. Buller | Staff Fellow | LPVD, NIAID |
| | L. H. Evans | Staff Fellow | LPVD, NIAID |

COOPERATING UNITS (if any)

Dr. A. Rein, NCI, Frederick, MD; Dr. J. A. Levy, Univ. Calif., San Francisco, CA; Dr. J. Kaplan, Univ. Utah, Salt Lake City, UT

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 2.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The goal of this project is to identify murine retroviral gene products that are expressed during development and their possible role in resistance to retrovirus-induced disease. Using a panel of monoclonal antibodies derived from mice undergoing graft-versus-host disease we have identified a viral gp70 in embryo cell cultures. This gp70 is serologically related to a group of pathogenic recombinant (dualtropic) viruses and appears to correlate precisely in mouse strain distribution with the Rmcf resistance gene. This gene has been identified in certain strains of mice which are resistant to some forms of retrovirus-induced leukemia and specifically confers resistance to replication of recombinant dualtropic viruses. We are currently carrying out genetic experiments to determine linkage between this endogenous viral sequence and the Rmcf locus on chromosome 5.

In order to gain a better understanding of the possible role of these endogenous viral sequences in resistance to disease, we have carried out some basic studies on the mechanisms of virus attachment and penetration of host cells. The efficiency of infection was found to be optimal at pH 7.6 but was markedly inhibited at mildly acidic pH. This was in striking contrast to the strict acidic pH dependence of entry of many other RNA enveloped viruses. We found that although the virus adsorption step was insensitive to pH, the rate of virus penetration was markedly inhibited at pH<6.4. The post-adsorption pH-sensitive step was a fusion event which was found to have an optimum of pH 7.6. These observations suggest that some murine retroviruses may be unable to enter the cytosol from within acidified endosomes, a common route of entry among other RNA viruses. Our current efforts in this area are focused on the identification of a retroviral fusion protein and its function in virus entry.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00199-06 LPVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Immunobiology of Aleutian Disease

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. E. Race | Veterinary Officer | LPVD, NIAID |

Others:

COOPERATING UNITS (*if any*)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 0.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to define immune mechanisms and viral characteristics important in the pathogenesis of Aleutian disease (AD). Monoclonal antibodies were used to study antigenic differences among strains of ADV and to characterize viral proteins in vitro and in vivo. Highly virulent Utah I ADV was clearly delineated from the tissue culture-adapted avirulent ADV-G strain. This specificity could be demonstrated by indirect immunofluorescence (IFA) against infected cultures of Crandell feline kidney cells or against tissues of Utah I ADV-infected mink. Immunoprecipitation analyses utilizing various mAbs identified specific antigenic determinants. When immunoprecipitation-defined reactivities were correlated with IFA tissue and in vitro patterns of reactivity it was apparent that the virus-associated antigenic determinants recognized in vivo were proteolytic products of viral structural proteins. Intact structural ADV proteins were not identified in vivo. However, structural proteins were detected in vitro when ADV-G or Utah I ADV-infected CRFK cells were analyzed. Thus, proteolysis occurred in vivo , resulting in small ADV related polypeptides but was not a significant finding in vitro where ADV structural proteins were the predominant viral antigen.

PHS 6040 Rev. 1/84

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00265-04 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R. E. Race | Veterinary Officer | LPVD, NIAID |
| Others: | B. Chesebro | Chief | LPVD, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 0.7 | 0.9 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeldt-Jakob and Kuru diseases of man. The infectious agent is markedly resistant to agents that inactivate most viruses and disease results in no detectable host immune response. The intimate association of scrapie with host protein may account for many of its unusual features. In order to obtain agent free of tissue contaminants, we have sought to establish high titered scrapie infected tissue culture cell lines.

Neuroblastoma cell lines were successfully infected with scrapie agent and analyzed by two-dimensional gel electrophoresis for scrapie-specific proteins and for the presence of the "prion" protein. No protein unique to either the uninfected or infected culture was identified. Attempts to metabolically label scrapie associated nucleic acids were also unsuccessful. Although the cell line maintains significant infectivity, it may be too little to allow detection of certain Sc related functions. Therefore, an attempt will be made to clone infected cells from the cultures thus enriching for Sc related functions.

In order to study the relation of the prion protein to scrapie disease a cDNA clone of the scrapie prion protein (PrP27-30) was isolated using an oligonucleotide probe based on the predicted mRNA sequence of a portion of this protein. The partial DNA sequence of this clone indicated that it was identical to the predicted PrP27-30 mRNA. Hybridization of this clone detected a 2.4-2.5 kb mRNA band in both scrapie-infected and uninfected mouse and hamster brain, but not in spleen or liver. Thus, PrP27-30 mRNA was not scrapie-specific, but instead appeared to encode a normal brain protein. Similar approaches will be used to search for scrapie specific markers.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00266-04 LPVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | L. H. Evans | Senior Staff Fellow | LPVD, NIAID |
| Others: | J. D. Morrey | Staff Fellow | LPVD, NIAID |

COOPERATING UNITS (*if any*)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The current objectives of this project are to identify and characterize the types of recombinants generated between ecotropic murine leukemia virus (MuLVs) and endogenous retroviral gene sequences. Analyses of recombinant polytropic viruses generated after inoculation of mice with different ecotropic MuLVs demonstrated that different MuLVs specifically recombine with particular endogenous retroviral sequences to generate recombinants. Polytropic viruses derived from different endogenous sequences exhibited different biological properties, including their oncogenicity and their in vitro host ranges. Analyses of polytropic viruses generated after inoculation of in vitro-constructed recombinants between ecotropic MuLVs which differ in their specificity of recombination have identified the 3' LTR of the viral genome as a region which influences the specificity.

AKR/J mice harbor endogenous ecotropic viruses and exhibit a high incidence of spontaneous lymphomas. Utilizing an immunofluorescence assay with monoclonal antibodies, two novel types of recombinant viruses have been identified in preleukemic AKR/J mice. Recombinant viruses were isolated from the spleens of mice as young as 1 month of age. These isolates possess antigens characteristic of polytropic viruses but exhibit a more limited in vitro host range. In contrast to polytropic viruses, they do not infect SC-1 (mouse) or mink lung fibroblasts, but are highly infectious for a *Mus dunni* cell line. At about 4 months of age, a second type of recombinant virus was identified in the thymuses. SC-1, mink and *Mus dunni* cells could only be infected with these recombinants by co-cultivation with thymocytes. These two types of recombinant MuLVs are the earliest and the most prevalent recombinants found in preleukemic mice and their role in leukemogenesis is under investigation.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 AI 00386-02 LPVD |
|---|---|

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Transformation of Hematopoietic Cells by Avian Tumor Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | S. Palmieri | Staff Fellow | LPVD,NIAID |
| Others: | None | | |

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two groups of viruses are being studied: isolates of Rous Sarcoma Virus (RSV) and those containing the myc oncogene (MH2, MC29, OK10, CMII). Studies have revealed that RSV has the capacity to transform erythroid cells both in vitro and in vivo but in a manner distinct from avian erythroblastosis virus (AEV). Since the erb B and src proteins encoded by AEV and RSV, respectively have been previously shown to share a significant amino acid homology, the present observation suggests that both may also share a common functional homology. With regard to the myc containing viruses, a conditional mutant of MH2 has been isolated which permits infected macrophages to differentiate at the nonpermissive temperature. The mutant was characterized in macrophage and fibroblast transformation assays as well as in vivo studies. By comparing the transforming properties of the ts mutant and wtMH2 viruses in these different systems, the ts mutation within the MH2 genome could be localized to a region of the myc gene which controls macrophage but not fibroblast transformation. Neither the gag-mil nor the myc proteins encoded by the tsMH2 virus appeared to have incurred any molecular weight changes compared to wtMH2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00418-02 LPVD

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Equine Infectious Anemia Virus, a Retrovirus Model for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | B. Chesebro | Chief | LPVD, NIAID |
| Others: | S. Carpenter | Staff Fellow | LPVD, NIAID |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
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| 1.2 | 1.2 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


The goal of this project is to study antigenic variation of equine infectious anemia virus (EIAV) with regard to possible mechanisms of viral persistence through avoidance of the specific immune response. A focal immunofluorescence assay has been used to expand and biologically clone a number of viral isolates in equine, feline and canine cell lines. In addition, viral isolates from sequential febrile periods have been isolated from infected horse blood, have been adapted to replicate in an equine dermal cell line and have been biologically cloned in that cell line. One of these field isolates, MA-1, is being used to infect a horse in order to generate antigenic variants in vivo.

Monoclonal antibodies are being developed in order to antigenically characterize the various viral isolates. A total of 34 clones has been found reactive with EIAV-infected cells by membrane and/or cytoplasmic fluorescence. Further characterization of these clones is ongoing. Virus isolates are also being compared by restriction enzyme analyses of Hirt supernatant fractions of infected cells.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1985 Annual Report
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1984, to September 30, 1985

## Introduction

The branch provides all services necessary to the professional staff in the pursuit of their investigations. Fiscal support includes budget management, procurement and initiating payments and follow up on financial obligations related to purchases, contracts, staff and official guest travel and expenses for conferences held at RML. Other support covers the following areas: personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparation, waste disposal including hazardous wastes and radioactive wastes, glassware cleaning, photography, animal rearing and care, motor pool, operation of power plant and full maintenance and minor laboratory renovations in every area except electronics.

On November 13, 1984 the responsibility for operation of the heating plant was assumed by a private contractor under A-76. The five government employees who were affected by this change were transferred into other units of the Operations Branch.

On January 2, 1985 the Electron Microscopy Section of the Operations Branch was disestablished and this group was established as the Pathobiology Section in the newly formed Laboratory of Pathobiology.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of $1,744,000. Payroll is not included in this figure. It covers only the purchase of supplies and minor equipment used in the operation of the laboratories. Timekeeping and submission of the payroll are also handled in this unit.

Personnel handles all actions and advises on personnel matters. This department is also charged with operation of the Job Training Partnership Act in association with the local Montana State Employment Office. Through the year, we have averaged one person on this program. The maximum time a person may spend on the program is two months. Hence, we are constantly interviewing and employing people under the program. Also handled by Personnel are persons under the following programs: Stay-in-School, Work Study, Student Volunteers, Visiting Program, and students studying for advanced degrees.

Most of the biological media used in the research laboratories is prepared in a special laboratory by a technician. Glassware is cleaned and sterilized in the glassware department for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The Animal Unit raises rats, guinea pigs, 15 strains of mice, 5 strains of hamsters, and a colony of microtus. They breed and raise approximately 100,000 animals a year. An additional 7,000 animals are purchased annually from outside sources, including horses, mink, sheep, rabbits, mice, chickens and hamsters. After rearing, care is provided for these animals while they are under experiment.

The Chief of the Branch is responsible for labor management work and administering the technical aspects of the A-76 contracts for Security, Custodial and Operation of the Power Plant with the respective private contractors. Security is provided in the form of a guard on duty every night. Custodial services are provided in five laboratory buildings daily except weekends and holidays. Power plant operation provides heat, steam, compressed air, vacuum and emergency power to the entire laboratory complex.

The maintenance department provides repair, service and renovation work in plumbing, electrical, sheet metal, carpentry, air conditioning and refrigeration, including ultra low temperature boxes. With the exception of electronic work, all maintenance is done by the staff. Also provided are demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care including snow removal is provided.

LABORATORY OF IMMUNOPATHOLOGY
1985 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF IMMUNOPATHOLOGY, NIAID
October 1, 1984 to September 30, 1985

Herbert C. Morse III, M.D.
Chief, Laboratory of Immunopathology

The Laboratory of Immunopathology was created in May 1985 through the union of the Viral Oncology Section and the Virology and Cellular Immunology Section from the Laboratory of Viral Diseases with the former Biology of Viruses Section from the Laboratory of Molecular Microbiology. Dr. Herbert C. Morse III, Head of the Virology and Cellular Immunology Section was named Laboratory Chief. Dr. Janet W. Hartley continues as Head of the Viral Oncology Section and Dr. Andrew M. Lewis is head of a new Viral Pathogenesis Section. The Heads of all three sections had earlier been members of Dr. Wallace P. Rowe's Laboratory of Viral Diseases and his influence on the direction of many aspects of their work continues.

The research interests of all three sections are focused, in major part, on the role of viruses in cell transformation and neoplasia. C-type murine leukemia viruses (MuLV) are the focal point of studies in the Viral Oncology and Virology and Cellular Immunology Sections while the functions of recombinants between SV40 and adenoviruses are the basis for studies in the Viral Pathogenesis Section.

Research conducted by members of the Virology and Cellular Immunology Section has indicated that neoplasms belonging to B cell and myeloid differentiation pathways are more closely related than was previously understood. Several distinct neoplasms were found to express characteristics common to both myeloid and B cells suggesting that the transformation event occurred in a precursor to both these cell lineages. In collaborations with the Viral Oncology Section, it was shown that greying with age in mice is due to the effects of MuLV expression during melanocyte development leading to a premature cessation of melanosome production. A new murine syndrome characterized by lymphoproliferation and severe immunosuppression and induced by a novel recombinant MuLV was described. This syndrome may prove to be an important model for understanding retrovirus-induced acquired immunodeficiency in man. Joint studies with the Viral Oncology Section have demonostrated that MuLV containing the _myc_ oncogene induce morphologic transformation of some continuous _in vitro_ cell lines and that infection of newborn mice with these viruses results in neoplasms of ectodermal, endodermal and mesodermal origin.

Studies in the Viral Oncology Section have centered on biological and molecular characterization of several new MuLVs of both the defective oncogene transductant type and replication competent MCF-type recombinant class, the former being _ras_ - containing hemangiosarcoma-erythroleukemia inducing agents and the latter lymphoma or leukemia inducing viruses generated during infection of mice with wild mouse ecotropic MuLVs. Further studies of _in vitro_ virus constructs prepared by exchanging genome segments of molecularly cloned MuLVs with differing disease inducing potential have indicated that the influence of specific putative viral transcription enhancing regions on dis-

ease phenotype varies from a dramatic reciprocal exchange of lymphomagenicity and erythroleukemogenicity in the case of Moloney and Friend MuLV recombinants to a more subtle or additive effect as with LTR U3 region exchanges between lymphomagenic AKR 247 MCF virus and erythroleukemia-inducing Friend MuLV.

Since Dr. Lewis' group has been part of this laboratory only since May 1985, report of his work is contained in the report of his former laboratory, Laboratory of Molecular Microbiology.

Highlights of the current year are as follows:

Relations of B cell and myeloid differentiation. A lymphoid cell line, P388, and a macrophage cell line, P388D1, derived from a single mouse were shown to have apparently identical immunoglobulin gene rearrangements and to express cell surface antigens characterisitc of both B and myeloid cells. Another lymphoid cell line, HAFTL3, was shown to spontaneously convert to a cell line with myeloid characteristics. These cell lines may help to explain some of the transitions between the myeloid and B cell differentiation pathways observed in chronic myelogenous leukemia (Holmes, Pierce, Bauer, Davidson, Morse).

Greying with age in mice. Certain strains of C57BL/10 mice congenic for different H-2 haplotypes were found to grey prematurely. Greying, but not non-greying strains, expressed high levels of ecotropic MuLV from early in life. The greying phenotype and high virus expression could be transmitted by foster-nursing of low virus mice on high virus mothers whereas greying, high-virus mice fostered on virus-negative mothers developed a low-virus, non-greying phenotype. Studies of skin biopsies from greying mice revealed dense accumulations of MuLV in the dermis indicating relationship between MuLV expression and greying (Morse, Yetter, Pitts, Stimpfling, Fredrickson, Hartley).

Mouse acquired immunodeficiency syndrome (MAIDS). Adult C57BL/6 mice infected with viruses recovered from a radiation-induced lymphoma developed massive, non-malignant lymphoproliferation associated with profound immunosuppression. Mice infected with this virus (LP-BM5) were incapable of mounting any B cell- or T cell-dependent responses, in vivo or in vitro, to a variety of antigens. The virus infected B cells and macrophages but could not be readily recovered from T cells. The MAIDS model may prove to be useful in vaccine and anti-viral chemotherapy studies related to human retroviral-induced immunosuppression (Yetter, Mosier, Morse, Fredrickson, Hartley).

The myc oncogene in neoplasia. Deregulation of myc expression is most commonly associated with B lineage lymphomas. Studies of mice infected with MuLV containing myc revealed T cell, B cell, pre-B cell and myeloid lymphomas/leukemias as well as pancreatic adenocarcinomas and other tumors. Altered expression of the myc oncogene thus, unexpectedly, results in transformation of cells from multiple lineages (Morse, Hartley, Fredrickson, Yetter, Cleveland, Rapp).

New transforming viruses contain ras oncogene. Two independently generated defective transforming viruses containing the ras oncogene have been isolated from v-congenic mice which had developed long latent period tumors following inoculation with a MCF virus; both isolates rapidly induce hemangiosarcoma and erythroleukemia. One virus has been molecularly cloned and by restriction mapping and nucleotide sequencing shown to be an ecotropic MuLV, 8.8 kb in length

and containing a 1 kb replacement replacing the gp70 region of env, consisting of the ras p21 coding region with a mutation to arginine at amino acid 12. The second virus is of similar structure and biological activity but lacks a non-coding sequence of 100 bp downstream of the p21 coding region (Fredrickson, Hartley, (LIP); O'Neill, Theodore, Rutledge, Martin (LMM)).

MCF virus efficient inducer of thymic lymphomas in NFS mice. Biologically cloned MCF viruses recovered from thymic lymphomas developing in mice inoculated with a wild mouse ecotropic virus, CasBr-M, vary in their pathogenicity. One isolate, NS-6(186) MCF, induces a high frequency of thymic lymphomas in ecotropic virus-free NFS mice within 2-3 months (Holmes, Morse, Hartley).

NS-6(186) MCF virus molecularly cloned. Biologically active molecular clones of the lymphomagenic NS-6(186) MCF virus have been obtained. Restriction enzyme mapping to date suggests that this virus is very similar to its putative parental wild mouse ecotropic virus except in the gp70 region of env. Selected nucleotide sequencing is in progress to determine precise differences from the ecotropic parent and other, non-oncogenic, MCF isolates of similar origin (Chattopadhyay, Hartley, Morse).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00135-11 LIP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Properties of immunoglobulin secreting cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI : | H. C. Morse III | Chief, Laboratory of Immunopathology | LIP, NIAID |
| Others: | W. F. Davidson | Visiting Scientist | LG, NCI |
| | J. W. Hartley | Senior Investigator | LIP, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LIP, NIAID |
| | K. L. Holmes | Staff Fellow | LIP, NIAID |
| | U. R. Rapp | Senior Investigator | FCRC, NCI |
| | R. A. Yetter | Guest Worker | LIP, NIAID |

COOPERATING UNITS (if any)

R.L. Coffman, DNAX Research Institute, Palo Alto, CA; U.R. Hammerling, Sloan-Kettering Cancer Center, New York, NY

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)


The development of B cells from the earliest committed precursors to mature secreting cells is marked, phenotypically and genotypically, by the sequential expression of a series of cell surface antigens and immunoglobulin gene rearrangements. One of the earliest markers expressed in the B cell and other hematopoietic lineages is the Ly-17 alloantigen. Phenotypic, functional and biochemical studies of antibodies to allelic specificities of Ly-17 showed that they recognize genetically-determined polymorphisms of the murine Ig gamma Fc receptor.

Analyses of B lineage lymphomas indicated that B cell development does not involve a single linear pathway of differentiation. Although all B cells may originate from a common precursor population, they appear to rapidly diverge into independent Ly-1$^+$ and Ly-1$^-$ pathways. A precursor cell for both these pathways may express the Mac-1 antigen indicating that the B cell and myeloid differentiative pathways are closer than appreciated previously.

Altered expression of the <u>myc</u> oncogene is associated with development of B-lineage neoplasms in mouse and man whereas expression of the <u>raf</u> oncogene is associated with development of sarcomas and erythroblastosis in mice. Mice infected with <u>raf/myc</u> - containing viruses developed not only sarcomas and erythroblastosis but pre-B, B and T cell lymphomas and pancreatic adenocarcinoma. The non-<u>raf</u>-like neoplasms were also induced with retroviruses expressing <u>myc</u> alone.




PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00138-11 LIP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Viruses and the immune response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI | H. C. Morse III | Chief, Laboratory of Immunopathology | LIP, NIAID |
| Others: | J. W. Hartley | Senior Investigator | LIP, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LIP, NIAID |
| | R. A. Yetter | Guest Worker | LIP, NIAID |
| | K. L. Holmes | Staff Fellow | LIP, NIAID |
| | J. N. Ihle | Senior Investigator | FCRC, NCI |

COOPERATING UNITS (if any)

P.M. Hoffman, V.A. Research Service at the University of Maryland, Baltimore, MD; J.H. Stimpfling, McLaughlin Research Institute, Great Falls, MT

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Greying with age in mice was shown to be due to effects of murine leukemia viruses (MuLV) on melanocyte function. Mice exposed to MuLV by day 8 of gestation developed patterned greying consistent with infection of melanocyte precursors. Mice infected at birth with MuLV developed diffuse greying which could be related to infection of more mature cells in melanocyte differentiation.

Induction of neurogenic paralysis by Cas-Br-M MuLV was examined to determine the role of host genes in the development of disease and to determine if immune responses were of importance in resistance to disease induction. Two or more unlinked autosomal dominant loci were shown to control the resistance of some mouse strains to disease. Other results suggest that these genes control the ability of Cas-Br-M to replicate in the central nervous system.

Retroviral induction of immunosuppression has gained much attention through the outbreak of AIDS in the United States. A murine retrovirus that induces lymphoproliferation and profound suppression of T cell and B cell responses has been defined as a component in the mixture of viruses recovered from a radiation-induced lymphoma.

Activation of the <u>myc</u> oncogene has been implicated in the development of B cell-lineage lymphomas in mouse and man. Mice inoculated with retroviruses containing avian v-<u>myc</u> developed B lineage lymphomas as well as T cell tumors, pancreatic adenocarcinoma, hepatic and pulmonary neoplasms. These results suggest that <u>myc</u> deregulation can affect the growth characteristics of multiple cell lineages.




PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00205-05 LIP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B lymphocytes of autoimmune mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
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| PI : | H. C. Morse III | Chief, Laboratory of Immunopathology | LIP, NIAID |
| Others: | W. F. Davidson | Visiting Scientist | LG, NCI |
| | J. W. Hartley | Senior Investigator | LIP, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LIP, NIAID |
| | R. A. Yetter | Guest Worker | LIP, NIAID |
| | K. L. Holmes | Staff Fellow | LIP, NIAID |
| | W. Y. Langdon | Visiting Fellow | LIP, NIAID |
| | E. K. Rudikoff | Biological Laboratory Technician | LIP, NIAID |

COOPERATING UNITS (if any)

J.B. Roths, Jackson, Laboratory, Bar Harbor, ME; W. Saunders, Howard University, Washington, DC; F. Dumont, Merk Laboratories, Rahway, NJ.

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Virology and Cellular Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.0 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


Mice bearing the non-allelic mutations lpr and gld develop massive lymphoproliferation and autoimmunity. Phenotypic and functional studies of lymphocytes from gld homozygotes showed that they had many abnormalities in common with mice homozygous for the lpr mutation. These two mutations may affect different enzymes in a single metabolic pathway of major importance to T cell development.

SJL/J mice homozygous for the lpr mutation were found to die with progressive lung disease and their lymphocytes expressed high levels of infectious ecotropic murine leukemia viruses. Mice of this strain heterozygous for lpr died with accelerated B cell lineage lymphomas. An effect of lpr in the heterozygous state on disease was not seen in other strains of mice. Enhanced virus expression in lpr mice was not related to linkage between lpr and one of the two endogenous ecotropic proviruses.




PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 AI 00284-04-LIP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of pathogenic murine leukemia viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI : | J. W. Hartley | Head, Viral Oncology Section | LIP, NIAID |
| Others: | T. N. Fredrickson | Research Microbiologist | LIP, NIAID |
| | R. R. O'Neill | Senior Investigator | LMM, NIAID |
| | M. A. Martin | Senior Investigator | LMM, NIAID |
| | S. K. Chattopadhyay | Visiting Scientist | LCO, NCI |

COOPERATING UNITS (if any)

Nancy Hopkins, MIT; Stephen Staal, Johns Hopkins University

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 1.3 | 1.7 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


This project comprises biological and molecular studies of various replication competent, non-oncogene containing ecotropic and MCF-type recombinant murine leukemia viruses (MuLVs), and hybrid MuLVs constructed *in vitro* by exchange of genome components of viruses with differing properties. Viral pathogenesis is studied in depth following inoculation of selected mouse strains, by testing of mice by a variety of techniques for replication of input virus and generation of new recombinant viruses, histopathological and immunological characterization of tumors, and molecular study of tumors for new proviral integrations or rearrangements of cellular genes, or for expression of activated gene products.

Of particular interest have been the ecotropic MuLVs isolated from California wild mice, viruses which can induce both a neurological disease and a variety of T-, B-, erythroid and myeloid cell neoplasms, and the MCF viruses recovered with high frequency from such tumors. In addition to delineating further the diverse disease inducing potential of the ecotropic viruses we have isolated and biologically characterized 3 new oncogene-containing viruses (2 representing transductions of activated mouse c-*ras*, and 1 containing an as yet unidentified oncogene), a highly lymphomagenic MCF virus which is helper-independent for both replication and induction of disease, and several other MCF viruses of potential interest. One of the *ras*-related viruses has been molecularly cloned and extensively characterized. Biologically active molecular clones have also been obtained for the lymphomagenic MCF virus and mapping and sequencing studies are in progress. Construction of hybrid virus genomes from portions of molecularly cloned viral DNAs has yielded viruses with altered pathogenic capacities. Efforts have concentrated on determining the genome segment(s) controlling target cell specificity as manifested by virus recovery and nature of the disease induced. These studies indicate major roles for the LTR region, specifically putative enhancer sequences in U3, in Friend MuLV-induced erythroleukemia and Moloney MuLV induced lymphoblastic lymphoma; and for LTR plus gp70, Prp15, and *gag* coding sequences in the case of AKR 247 MCF MuLV thymic lymphomagenesis.





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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-04 LIP

PERIOD COVERED

October 1, 1984 to September 30, 1985

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of genetic control of murine leukemia viruses and virus-induced neoplasms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | J. W. Hartley | Head, Viral Oncology Section | LIP, NIAID |
| Others: | H. C. Morse | Senior Investigator | LIP, NIAID |
| | T. N. Fredrickson | Research Microbiologist | LIP, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunopathology

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 0.5 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)


As part of a long-term program to analyze the effects of murine retrovirus associated genes on hematopoietic system tumors, both spontaneous and those induced by chemicals or virus inoculation, a number of genes of interest are being bred onto inbred mouse backgrounds. The majority of congenic lines are being established on the NFS Swiss mouse background, providing a standard background which is negative for its own endogenous ecotropic virus. Lines include the V-loci congenics (carrying ecotropic virus loci from AKR, C58, or C3H/Fg; and mice carrying specific viral resistance genes (Fv-4, Rmcf) or linkage marker genes. Two attempts are in progress to establish congenic lines of AKR and C57BL which will lack ecotropic MuLV induction loci; these lines are at backcross 8 and backcross 6, respectively.

V-congenic mice, particularly NS.C58v1, are being used in studies of oncogenicity of various acute transforming and replication competent MuLVs.